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STUDIES ON COLLAGEN

I. Structure of the Collagen Group of Proteins

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1. Introduction

The collagen group forms an important subdivision of the fibrous proteins. While the main details of the structure of the keratin group and of silk fibroin are now fairly well understood (Pauling and Corey, 1953; Crick, 1953 *a*; Marsh, Corey and Pauling, 1955), the same cannot be said of the collagen group. Several structures have been proposed for collagen [for a review of work prior to 1952, see the article by Bear (1952)], but none of these are in agreement with the available data on all the various properties of collagen. More recently, structures have been proposed for collagen by Randall, Fraser and North (1953), Crick (1954) and Huggins (1954). These have also not proved to be completely satisfactory.

A structure was put forward from this laboratory about a year ago (Ramachandran and Kartha, 1954). This fits the infra-red and chemical evidence on collagen, but is found to be in disagreement with the latest X-ray data. Cowan, North and Randall (1953) showed that by stretching collagen fibres, the X-ray pattern revealed much more detail than was ever obtained previously. The pattern shows clearly that the structure of collagen is based on a helical arrangement. An approximate measurement of the layer line spacings indicates that the helix may have 7 units in about 20 A along the fibre axis, or 10 units in about 30 A. The former choice was favoured by Cohen and Bear (1953), but the latter was shown to fit the X-ray diagram much better by Ramachandran and Ambady (1954), who also indicated that the radius of the primary helix is of the order of 1 A. It has now been possible to work out the details of the structure satisfying the above specifications demanded by the X-ray data and also taking into consideration evidence from other directions, such as infra-red absorption, chemical composition and reactivity, and so on. This structure turns out to be only a slightly modified form of the earlier structure (Ramachandran and Kartha, 1954) and preserves most of its features. A preliminary account of the structure has been published in *Nature* (Ramachandran and Kartha, 1955).

2. Review of Data on Collagen

In order to appreciate how far the proposed structure helps in understanding the nature and behaviour of collagen, we shall give a brief summary

of the available data on collagen. In view of the excellent review published by Bear in 1952 and the discussion given by Kendrew (1954) in the treatise *The Proteins*, Vol. II B, attention will be directed here only to the salient features which have a bearing on the discussions in later sections.*

(*a*) *Chemical data.*—The amino-acid composition of collagen from different sources exhibits a remarkable uniformity (Tristram, 1953). Approximately one-third the number of residues are glycine (G, say), another one-third are composed of proline and hydroxyproline (P, say) residues, while the remaining one-third comprise the other residues (R, say). In general, the G-residues are slightly more than one-third while the P-residues are less than one-third. The relative proportion among the P-residues of proline and hydroxyproline is variable (from about 1 : 1 to 2 : 1). While mammalian collagen has a high hydroxyproline content (about 14 per cent.), it is as low as 4 per cent. in some fish collagens, and the thermal stability of collagen is found to go hand in hand with the hydroxyproline content (Gustavson, 1954, 1955). Apart from alanine, which forms about 30 per cent. of the R-type residues, there are very few non-polar residues. Most of them are polar, with both acid as well as basic side-groups, the more important ones being arginine and lysine, and aspartic and glutamic acids.

The sequence of amino-acid residues in collagen has been studied by various workers, the latest being those of Schroeder and co-workers (1953, 1954). A number of sequences of two and three residues have been identified in the hydrolysate. They do not appear to support the repeated occurrence of the sequence-G-R-P-. On the other hand, there seems to be definite evidence that the sequence gly-pro-hypro is of frequent occurrence.

There seems to be no definite evidence that *cis*-type of residues occur in collagen. On the other hand, there is reason to believe that they do not occur (Badger and Pullin, 1954).

(*b*) *Infra-red.*—Ambrose and Elliott (1951) observed that the infra-red dichroism of the CO and NH stretching vibrations could be explained satisfactorily if these bonds were at right angles to the fibre axis. Although there is some doubt whether the direction of the induced moment is exactly parallel to the direction of vibration (Price and Fraser, 1952), the angle between the two cannot be large. Thus, the infra-red data indicate that the CO and NH bond directions are nearly perpendicular to the fibre axis. Recently,

* After this paper was prepared in the final form, Symposium No. 9 of the Society for Experimental Biology (Cambridge University Press, 1955) reached the authors. The results reported therein are not considered here in detail.

Sutherland, Tanner and Wood (1954) have recorded data on the dichroism of other bands in the infra-red spectrum of collagen fibres.

(*c*) *X-ray diffraction.*—An important advance in our knowledge of the high angle diffraction pattern was made by the recording of the pattern of stretched collagen by Cowan, North and Randall (1953). It is now clear that the 2·86 A arc found in unstretched collagen is a true meridional reflection, while the 4 A arc is composed of two non-meridional reflections (see also a succeeding paper by Ramachandran and Ambady in this series). A number of other spots have also been recorded, which occur on layer lines, whose spacings are not integral multiples or submultiples of the prominent layer lines at 2·86 A, 4·1 A and 9·5 A. The high angle diagram shows clearly that the structure is helical in nature, and that the resolved component of the length of an amino-acid residue along the fibre axis is 2·86 A in unstretched collagen which may increase to as much as 2·95 on stretching. Several possible values have been given for the details of the helix, such as 7 residues in 2 turns or 10 residues in 3 turns (Cowan, North and Randall, 1953; Ramachandran and Ambady, 1954; Cohen and Bear, 1953; Randall, 1954 *a*). A larger repeat distance along the fibre axis is not ruled out.

The small angle pattern indicates the existence of an axial period of 640 A in dry collagen, which has been confirmed by electron microscopy. The variation of this with stretching does not follow the corresponding variation of the 2·86 A spacing (Cowan, North and Randall, 1954 *a*), but the details are not available. There is also some evidence for the existence of a sub-unit of dimensions 220 A along the fibre axis (North, Cowan and Randall, 1954). The variation of the small angle pattern with moisture content has been studied in detail by Rougvie and Bear (1953), who find in general an increase of the long spacing with increasing humidity. Pronounced changes have been observed in the intensity distribution in the small angle pattern as a result of tanning (Bolduan, Salo and Bear, 1951).

The unit cell dimensions (*a* and *b*) of collagen in the plane perpendicular to the fibre axis (*c*) have not been definitely established. Two suggestions have been made: (*a*) originally due to Astbury (1940) of taking the spacing of the principal equatorial spot at 11–15 A (varying with moisture content) as one and the diffuse spot at 4·5 A as the other and (*b*) due to Pauling and Corey (1951) of taking the unit cell as hexagonal, identifying the values 11–15 A as the (100) spacing. The principal objection to the latter has been that the spot corresponding to the 110 reflection has never been observed on the equator, even though the value of *a* changes by as much as 50 per cent.; nor has a spot having this ξ-value been previously observed in

any of the other layers. Recently, Ramachandran and Ambady (1954) have recorded such a spot in the layer of spacing 7·2 A, which supports the Pauling-Corey hexagonal cell.

The choice of the unit cell is closely related to the density of collagen. Bear (1952) has concluded that, with Pauling-Corey unit cell, three residues per unit cell occurring in an axial projection of 2·86 A would fit the observed density reasonably well.

Very recently, the cylindrical Patterson function of collagen, computed from its X-ray diagram, has been published (Yakel and Schatz, 1955).

(*d*) *Electron microscopy*.—The spacing of 640 A found in the small angle X-ray pattern has also been observed in electron micrographs of collagen fibrils (for details, see Bear, 1952). The details within this period have been studied, and it is found that in general 6 or 7 bands occur within the main repeat. The widths of these sub-bands have been measured, and although they are not all equal, they roughly subdivide the main spacing into 6 equal parts of spacing approximately 100 A. By a study of the micrographs given by stained and tanned fibres, it has been concluded that the stain is preferentially taken up in certain regions within the main period of 640 A and that other regions are relatively unaffected—called " bands" and " interbands " by Bear. The 6-fold subdivision is thus clearly seen in chrome tanned calfskin, stained with PTA (Schmitt and Gross, 1948).

A number of modifications of collagen have been detected by the electron microscope, mainly by Highberger and associates (Highberger, Gross and Schmitt, 1950; Schmitt, Gross and Highberger, 1953). Two distinct types have been discovered, the so-called fibrous long-spacing or FLS fibrils and the segment long-spacing or SLS fibrils. Both have a period of the order of 2000 A, but while the band system in FLS fibrils are non-polarised and small in number, the bands in the SLS material is polarised, and further the fibrils aggregate side by side, forming sheets or segments. The precise conditions under which these modifications are transformed into one another are not exactly known.

3. DETERMINATION OF THE STRUCTURE

Before describing the proposed structure, it may be worthwhile to mention the steps which led to its formulation. The earlier structure was worked out mainly on the following considerations:—

(*a*) The perpendicular dichroism of the CO and NH bands in the infrared, which indicate that these bonds are nearly at right angles to the fibre axis.

(*b*) The fact that more than one-third the number of residues in the protein are glycine residues.

It was found that these features could be explained by a structure based on a triple chain of residues, each chain being a helix composed of three residues per turn, having the symmetry 3_2. Both 3_1 and 3_2 are permissible; only 3_2 is possible if the amino-acid side-groups should have the L-configuration. The essential basis of the three chain protofibril is shown in Fig. 1, in which the individual chains may be denoted by the symbols A, B and C. The

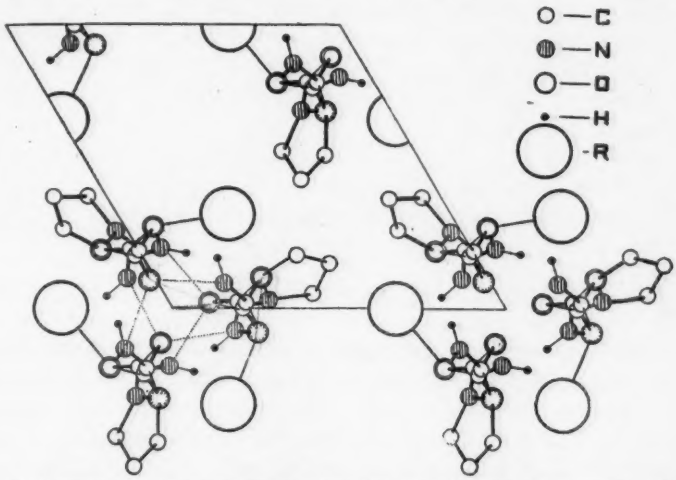

FIG. 1 (*a*) Projection of the structure along the *c*-axis. Two triple-chain rods and the contents of one unit cell are shown. The helices are right-handed (3_1). If the amino-acid residues have the L-configuration, they must be left-handed.

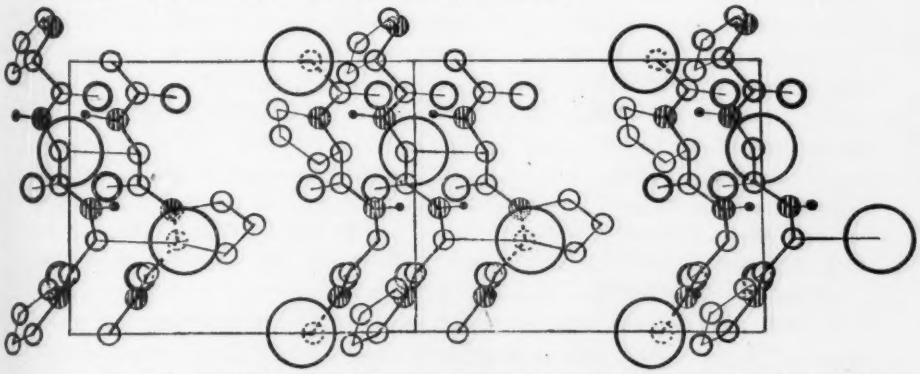

FIG. 1 (*b*). Projection along the *a*-axis. The *c*-axis is vertical in the figure. The contents of two unit cells are shown. Note the almost close packing of the atoms.

three chains are themselves related to each other by the operations of the symmetry element 3_2 parallel to the fibre axis through the centre 0. Thus the α-carbon atoms C_1 in the three chains are displaced successively along the fibre axis through a distance equal to one-third the pitch of the individual chains.

In actually working out the structure, the dimensions of the bonds and bond angles in the planar amide group were taken to be the same as those given by Corey and Pauling (1953). The angle at the α-carbon atom was taken to be equal to the tetrahedral angle and the two planar groups on either side of an α-carbon atom could rotate freely about the bonds joining them to this atom. In view of the rigidity of the backbone linking one α-carbon atom to another and the free rotation possible about the bonds at this atom, it is convenient to take the atoms in a link from one α-carbon atom to the next as a unit and denote them by the same subscript. The three α-carbons in one turn of each chain will be denoted by C_1, C_2, C_3, while the carbonyl carbon atoms will be distinguished by primes as C_1', C_2', C_3'. Thus, the sequence of atoms in the backbone will be:

$$-C_1(HR)-C_1'O_1-N_1H_1-C_2(HR)-C_2'O_2-N_2H_2-$$

If it is necessary to specify the chain to which an atom belongs, this will be done by adding an extra suffix indicating the nature of the chain: thus C_{1A} or C_{2B}. It is obvious that a single residue in the chain will be of the type $-N_1H_1-C_2(HR)-C_2'O_2-$. This residue would be referred to as the residue 2, the index corresponding to the suffix of the α-carbon atom.

The basal projection in Fig. 1 (*a*) shows clearly that the nearest neighbours of an α-carbon atom C_1 are the carbonyl oxygens O_2 of the other two chains. These are so close that there is no space for the hydrogen atom attached to C_1 to be replaced by any other group (not even by a methyl group). Further, the carbon atom C_3 could readily form part of the five-membered ring of a proline or hydroxyproline residue and the ring will be pointing outwards from the rod. It is also possible for the five-membered ring to be formed with the α-carbon atom C_2, with some strain on the bond at this atom. In the earlier note (Ramachandran and Kartha, 1954), this was not mentioned. It was then supposed that both proline and hydroxyproline residues occur only associated with C_3 and that this might explain the fact that these two together approximate to one-third the total number of residues. However, there now appears to be no reason why a proline residue may not occur next to glycine. This will be considered again later. Both NH and CO bonds are very nearly at right angles to the fibre axis in this structure.

An approximate agreement with X-ray data could be obtained by taking the 3 A arc to be non-meridional, and assuming that the length of three residues along the fibre axis is about 10 A. (For convenience of reference, the two principal arcs near the meridian will be referred to as the 3 A and 4 A arcs and the first strong layer line as the 10 A layer line.) However, by the time the details of this structure were worked out, the pattern of stretched collagen published by Cowan, North and Randall (1953) came to the notice of the authors. This showed clearly that the 3 A arc is truly meridional, while the 4 A arc is composed of two non-meridional spots. Attempts were then made in this laboratory to study this pattern in detail and the results are described in another paper in this series by Ramachandran and Ambady. By taking pictures with the stretched fibre set at the appropriate angle to the X-ray beam, the meridional reflection at 3 A could be obtained in great intensity, while the spots in the 4 A layer line clearly broke up into two. It was found that the pattern could be fairly well explained in terms of a helical structure having ten residues in three turns, with the radius of the helix of the order of 1 A (Ramachandran and Ambady, 1954). The order of the radius estimated for the helix was the same as that in the earlier structure, and this was strongly suggestive of the essential correctness of the earlier structure. However, it had to be modified to have three and one-third residues per turn, instead of three residues per turn. The dimensions of the helix, as well as the atomic co-ordinates were calculated for this helix with ten residues per three turns, having a projected length of 2·86 A per residue along the axis. An approximate calculation of the Fourier transform of this helix showed that the strong layer lines would be the third, seventh and tenth, as actually found.

An attempt was then made to put three of these helices side by side so that they are linked together by NH- - -OC′ hydrogen bonds. A number of trials were made, varying (*a*) the orientation of the chains by rotating all the three helices A, B, C simultaneously about their axes and (*b*) shifting them up and down along the common axis, but they were all unsuccessful. However, a satisfactory solution to the problem was found by coiling all the three individual helices about a common axis. This is to be expected, for helices having non-integral number of links per turn will fit only if they are wound into a superhelix. The main difficulty in the earlier attempts was the fact that as many as *ten* different types of hydrogen bonds had to be considered, of which at least six must be of the right length, if most of the free NH groups in the amino-acid residues must form hydrogen bonds. On the other hand, by forming the coiled coils, the number of different types of residues is reduced to three and only two types of NH....O bonds are

formed, assuming of course that the three individual chains are suitably displaced, so that they are symmetrically disposed with respect to one another. The extent of coiling about the axis of the cylindrical rod to achieve this is readily calculated. It turns out to be exactly one-tenth of a turn of every three residues, but in a direction opposite to that of the minor helix. [We shall use the nomenclature of Crick (1953 *b*) and call the helix of radius about 1 A the minor helix and the coil into which this helix is wound the major helix.] Thus in one complete turn of the major helix there are exactly thirty residues per turn, but as a result of the additional coiling, it makes ten turns for the thirty residues. Thus, with respect to the centre of the major helix, every third residue is in an equivalent position (*e.g.*, the links marked 1, 4, 7.... in Fig. 2).

Actually, there is not just one, but three chains (minor helices) and each of them is coiled further along the pattern of the major helix. Given the

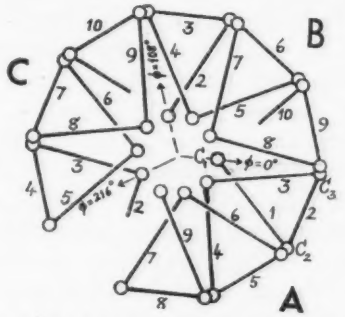

FIG. 2. Arrangement of coiled coils. Only the positions of the α-carbon atoms in each link are shown by open circles. The three chains A, B, C start at $\phi=0$, $z=0$; $\phi=108°$, $z=2{\cdot}86$ A and $\phi=216°$, $z=5{\cdot}72$ A. The major helix is left-handed and the minor helix is right-handed.

disposition of one of the chains, the arrangement of the other two readily follows from the fact that all the three are symmetrically disposed with respect to one another. This can be done by having the B chain to start with the α-carbon atom C_1 at the co-ordinates $\phi = 108°$, $z = 2{\cdot}86$ A and the C chain at $\phi = 216°$, $z = 2\times2{\cdot}86$ A. Proceeding one more step, the A chain will have an α-carbon atom at $\phi = 324°$, $z = 3\times2{\cdot}86 = 8{\cdot}58$ A, which is already there.

An approximate attempt at model building showed that good hydrogen bonds could be formed with such an arrangement if the radius of the major helix is close to 2·5 A. Precise drawings were then made with the major helix radius equal to 2·5 A. It is obvious that the length of the minor helix

per residue will no longer be 2·86 A if this is wound into a superhelix such that the projected length per residue along the axis of the major helix is 2·86 A. The former could readily be calculated, if the radius of the major helix is known, and is found to be equal to 2·91 A, when this is 2·5 A. The co-ordinates of the atoms in the minor helix with three and one-third residues per turn and a projected length of 2·91 A per residue were therefore calculated using the Pauling-Corey parameters for the amide group and taking the angle between the N–C and C–C′ bonds at the α-carbon atom close to $109\frac{1}{2}°$. The coiled structure was deduced from this by geometrical methods from the above considerations. The projection along the axis of the major helix was geometrically plotted, while the *z*-co-ordinate along this axis was calculated from the calculated inclination of the axis of the minor helix to the fibre axis. In both these processes, particularly in the former, it was necessary to assume that the twist induced by the second coiling at a carbonyl oxygen or the imino hydrogen atoms was the same as that at the corresponding carbon or nitrogen atoms. This is reasonable, since it is the latter which form the real backbone of the structure. An alternative was possible, namely, of finding the co-ordinates of the α-carbon atoms in the coiled coil and then putting in the other atoms in the planar Pauling-Corey configuration about the line joining these, as was done for the first non-coiled coil. The computational difficulties were however so large that only the geometrical method indicated above was adopted. The error introduced thereby is not expected to exceed about 0·1 A. In the case of the *z*-co-ordinates, the errors would be even less, as the NH and CO bonds were nearly perpendicular to the axis of the minor helix.

This still left one more degree of independence, namely, a rotation of the minor helix about its own axis (clearly all the three helices A, B, C have to be rotated by the same amount in the same direction). The trial was first made with the α-carbon atom C_1 as far inside the cylindrical rod as possible (as shown in Fig. 2) and the minor helices were then rotated upto $\pm 15°$ to either side. The best hydrogen bonds were formed (two for every three residues) very close to 0°, so that the first position was adopted. The radius of the major helix was then slightly varied, and the effect on the following were studied:

(*a*) the lengths of the two types of NH....O bonds, and the angles between the corresponding NH and NO directions.

(*b*) The distance between the α-carbon atom C_1 of each chain and the two closest oxygen atoms of the other two chains.

The former was considered to be the more important of the two criteria.

It must be mentioned that the essential feature of the structure is that every third residue in each of the three chains occurs rotated through an angle of 36° about the axis of the major helix and displaced parallel to it through a distance of 2·86 A. It is not essential that the three residues within this unit must conform to the pattern of a true coiled coil. All the essential features of the structure would still be retained even if there be a small deviation from the exact coiled-coil arrangement. Various types of deviations could be imagined, but the one actually tried out was to keep the α-carbon atoms in the same positions as in the exact arrangement, but to rotate the planar residues about the αC- αC-direction through a small angle. This would alter the angle between the N–C and C–C′ bonds at the α-carbon atoms. This angle was allowed to differ from the tetrahedral angle by as much as 3° and the best arrangement for the NH....O hydrogen bonds was studied.

The arrangement finally chosen was one in which the planes of the three amide groups make angles of 70°, 80°, 90° with the vertical plane through the α-carbon atoms at their two ends. Although the above arrangement does not form a true coiled coil, it still has an exact helical distribution of ten repeating units in three turns about the fibre axis, occurring in a distance of 28·6 A along the axis. Strictly, it is a triple helix with ten units occurring in one turn in a projected distance of 85·4 A along the axis, but the occurrence of three symmetrically disposed chains reduces the true repeat distance along the fibre axis to 28·6 A. The variations mentioned earlier were repeated with the new arrangement and the NH....O hydrogen bonds were both made to be approximately 2·8 A long. The major helix radius was finally fixed at 2·5 A.

The two NH....O distances were 2·87 A and 2·88 A. The distances from an α-carbon C_1 to the oxygen atoms nearest to it were 3·5, 3·15, 3·05 and 2·65 A. The last is considerably smaller than the carbon-oxygen Van der Waals contact distance. On the other hand, if the radius of the major helix is made larger, then the NH....O hydrogen bond lengths were increased too much, although the carbon-oxygen distance was satisfactory. If one of the hydrogen bonds is made short, while the other is comparatively long and not at the correct angle, then it is possible to avoid short carbon-oxygen distances; but this arrangement was not considered to be satisfactory.

4. DESCRIPTION OF THE STRUCTURE

The finally chosen co-ordinates of the various atoms in the backbone of the structure is given in Table I. The portion of the structure between the level $z = 0$ and $z = 8·58$ A is shown in Figs. 3 and 4. Figure 3 contains

TABLE I

Co-ordinates of atoms in one set of three residues. Cylindrical polar co-ordinates are given with respect to centre of major helix as origin

Atom	r in A	ϕ in degrees	z in A
Backbone			
C_1	0·98	0·0	0·00
C_1'	2·04	− 9·5	1·05
O_1	2·91	+10·5	1·41
N_1	2·51	−37·8	1·57
H_1	2·69	−60·1	1·30
C_2	3·50	−33·5	2·63
C_2'	2·89	−20·7	3·83
O_2	1·71	−12·0	3·95
N_2	3·76	−15·0	4·76
H_2	4·75	−17·0	4·73
C_3	3·50	− 1·6	5·95
C_3'	2·50	−16·5	6·78
O_3	2·66	−45·0	6·47
N_3	1·91	0·8	7·77
H_3	2·41	23·2	8·05
C_4	0·98	−36·0	8·58
Hydroxyproline side-chain			
C_5	4·83	− 1·9	6·71
C_6	5·90	−10·0	5·92
C_7	5·25	−17·5	4·74
O_4	6·93	− 3·0	5·48
H_4 (C_6)	6·35	−12·0	6·51

all the various atoms, while Fig. 4 is drawn with the purpose of showing the essential features of the structure and contains only the α-carbon atoms and the N and O atoms which are linked by hydrogen bonds. The co-ordinates are not considered to be final, but it is unlikely that they will have to be appreciably altered. Further refinement is difficult to make by calculation; precise models may be more useful for this purpose.

It will be noticed that the three chains together form a cylindrical rod, which may be considered to be the protofibril of collagen. As in the earlier model, every third residue in each chain is in a similar position as far as the backbone of the structure is concerned. In fact, every third α-carbon atom (type C_1) occurs on the surface of a cylinder of radius 9·5 A. It is not possible for the hydrogen attached to this atom to be replaced by a larger group and so every third residue must be a glycyl residue. The hydrogen bond formation in the new structure is slightly different from and more satisfactory

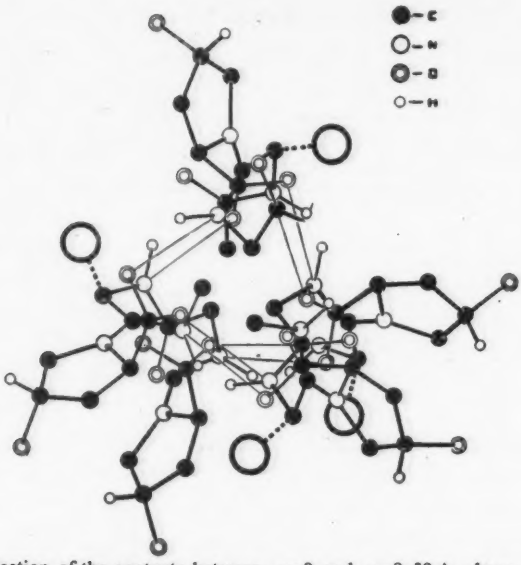

FIG. 3. Projection of the contents between $z=0$ and $z=8{\cdot}58$ A along the c-axis. The arrangement of the coils follows the scheme shown in Fig. 2. The hydrogen bonds are shown by thin lines. Refer to Table I for the co-ordinates of the atoms.

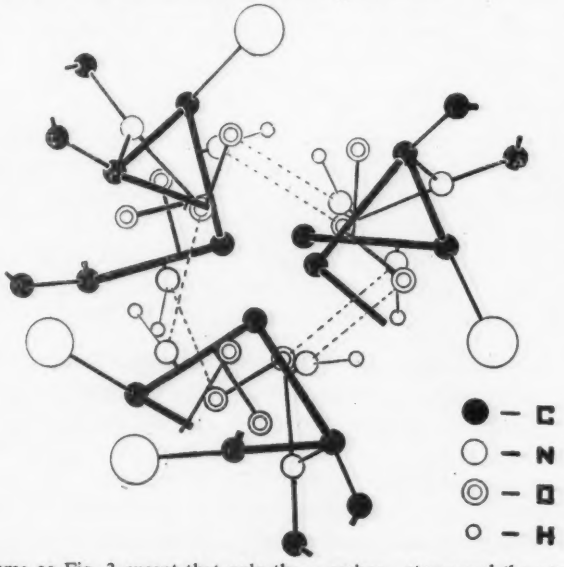

FIG. 4. Same as Fig. 3 except that only the α-carbon atoms and the atoms involved in hydrogen-bonding are shown. The links are indicated by straight lines, and their arrangement may be compared with Fig. 2.

than in the earlier model. The hydrogens in two of the three NH groups are linked to different oxygen atoms. The angles between the NH and N....O directions for the two types of bonds $N_1....O_2$ and $N_3....O_3$ are about 30°. These angles will be reduced further if the hydrogen atom is not coplanar with the rest of the atoms in the amide group. The third NH bond points out from the cylindrical rod and the side-chain of the corresponding residue will also extend outwards from the centre of the cylindrical unit. The pyrrolidine ring of a proline or hydroxyproline residue could conveniently occur in this position, and is marked as such in Fig. 3. The ring has been assumed to be planar and the C–OH bond is taken to be at the tetrahedral angle to the other two bonds of the same carbon atom in the plane of the ring. Although every third residue has been shown to be a hypro residue in this diagram, actually only part of them may be of this type.

Of the three types of carbonyl oxygen atoms, O_2 and O_3 are involved in internal hydrogen bond formation, while O_1 occurs on the outer surface of the cylindrical rod and cannot serve for this purpose. It is therefore available for forming external hydrogen bonds with other cylindrical rods by linking with a side-group.

For the same reason, it is obvious that the sequence of residues should exhibit a sort of periodicity, with every third residue being of the same type. One such regularity has already been noted, namely, the occurrence of a G-type residue once in every three residues. It had been assumed in the earlier model that the sequence of residues was of the type –G–R–P–G–. This however is not necessary. We shall now modify it to be of the type $-G-R_2-R_3-G-$. As mentioned earlier, the five-membered ring of a proline or hydroxyproline residue could be attached to either the C_2 or C_3 carbon atom. However C_3 will be favoured, as the corresponding NH group is not hydrogen-bonded. The choice of the location of the proline or hydroxyproline residues as well as the distribution of the other residues cannot be determined from a study of the backbone of a protofibril, but rather by a discussion of their cross-linkages. As will be shown below, the hydroxyl group of a hypro residue will be in the right position to form a hydrogen bond with the carbonyl oxygen O_1 of the neighbouring rod, if it occurs as the residue R_3. On the other hand, a proline residue occurring in this position serves no such purpose. Consequently, the proline may occur in position R_2.

Thus, the next step is to determine the relationship of the triple chain cylindrical rods to the lattice of the structure. As a first approximation, the lattice may be taken to be hexagonal, with the helix axis parallel to *c*,

which is the most reasonable arrangement of approximately cylindrical rods. The value of a for well-dried collagen has been found to be 12·0 A (= 10·4/0·866, Rougvie and Bear, 1953). Now, the oxygen of the hypro hydroxyl group extends to a distance of 6·93 A from the centre of the rod and its z-co-ordinate (5·48 for the residue A 2) is nearly the same as that of the non-hydrogen-bonded carbonyl oxygen at $z = 4{\cdot}27$ (O_1 of chain B). It is reasonable to suppose that these two atoms in neighbouring rods would be linked by a hydrogen bond and that the orientation of the cylindrical rod with reference to the lattice will be such as to make the length of this bond a minimum. If the hydrogen bond distance is made equal to 2·85 A, then the distance between the two cylindrical rods comes out as 11·4 A. This is seen to correspond roughly to the minimum value of a found for thoroughly dry kangaroo tail tendon.*

However, this simple arrangement does not ensure that a large number of hypro residues will be hydrogen bonded *via* their hydroxyl groups to their neighbouring rods. This is because the screw symmetry of the coiled coil does not fit in with the hexagonal symmetry of the lattice. However, a very slight alteration in the second coiling will be sufficient to satisfy this condition. The positions of the G-type α-carbon atoms in one chain are shown by dots in Fig. 5 in the natural position. The atom marked 37 has now to be moved anticlockwise through 12°, so that it has a value $\phi = 60°$. Then, if the hypro side-chain attached to the R_3 type residue belonging to the series starting from 1 can form readily a hydrogen bond with a carbonyl oxygen atom of its neighbour, so can the corresponding hypro side-chain belonging to the series beginning from 37. As will be seen from the figure, this only involves a very slight untwisting of the coiled coil. The twist for three residues is changed only from 36 to 35° and it is reasonable to assume that the structure could readily accommodate this change in order to form a large number of cross-links.

This condition, namely, that a large number of cross-linking hydrogen bonds are formed between the hydroxyl groups of hypro residues and carbonyl oxygens of a neighbouring rod, automatically introduces also a long-spacing for collagen. As shown in Fig. 5, the α-carbon 37 is at an angle $\phi = 60°$, so that α-carbon atom 217 will be exactly above the atom numbered 1. Thus, the repeat distance along the fibre axis is $72 \times 8{\cdot}58 = 618$ A. This is close to the value found for thoroughly dried unstretched kangaroo tail tendon (Rougvie and Bear, 1953).

* A value of 11·75 A, corresponding to the value of 10·15 A for the (100) spacing has been reported by Cowan, North and Randall (1955) with rat tail tendon.

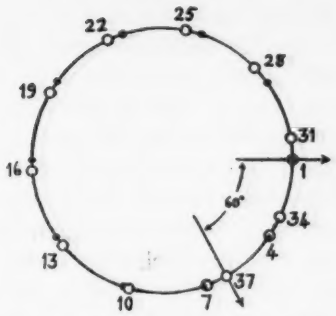

FIG. 5. Change produced in the coiling of the major helix in fitting the structure in a hexagonal lattice. Carbon atom 37 occurs at $\phi=360° + 60°$ instead of $\phi=360° + 72°$.

The distances from the hypro oxygen atom to a carbonyl oxygen in a neighbouring rod were measured for all possible positions for the hypro side-chain. The distances were measured graphically, corresponding to a value $a = 11{\cdot}4$ A for the lattice. It was found that in 12 out of the 36 possible positions the value was between 2·85 A and 3·15 A, while for the rest, it is longer. Thus, if we assume that a hypro residue occurs wherever it can readily form a hydrogen bond cross-link, then the corresponding percentage of hypro residues in collagen comes to be $100 \times 1/9$ or 11 per cent.

5. Comparison with Known Data on Collagen

(i) *Amino-acid composition and sequence of residues.*—The structure explains in a natural manner the fact that slightly more than one-third the total number of residues are glycyl residues. The structure demands further that every third residue shall be a glycyl residue. This is found to be in good accord with observation, since no sequence of three residues has been observed in the hydrolysate of collagen (or gelatin), which does not contain a glycyl residue (Schroeder *et al.*, 1954 and the earlier results quoted therein).

Further, the hypro residue* must occur in the position R_3 in the sequence $-G-R_2-R_3-$ according to the structure. Thus, the sequence hypro-gly must be of very frequent occurrence. It is interesting to note that of all the peptides isolated by Schroeder *et al.* (1954) which contain hydroxyproline, more than 95 per cent. has this sequence. So also, a good percentage of peptides having other hydroxy-amino-acid residues such as threonine and serine have the sequence threo-gly and ser-gly (90 and 85 per cent.). Thus it appears that all the residues having a terminal OH group in their side-chain occur in the position R_3. This again is reasonable, for they could

* We shall use the standard abbreviations for the amino-acid residues.

then readily link with a CO group of an adjacent rod by forming hydrogen bonds. The lengths of the side-chains in threonine and serine are much less than in hydroxyproline, but in the wet state (in which the collagen fabrils are built up), these groups could readily link to a carbonyl oxygen *via* a water molecule. Tyrosine residues should also occur in the position R_3 for the same reason, but no definite chemical evidence appears to be available. Further evidence that the hydroxylic residues occur as a group in the position R_3 is provided by a comparison of the amino-acid composition of fish skin and mammalian collagen (Tristram, 1953, p. 222). In fish skin, the hydroxyproline content is appreciably less, about 9 per cent. as compared with 14 per cent. for mammalian collagen, but the percentage of serine and threonine both go up appreciably, enough to make the total number of hydroxylic residues to be practically the same in both.

The position of proline residues is more difficult to fix from a consideration of the structure. As mentioned earlier, there is no difficulty in accommodating these residues in the position R_2 with a small strain and the data of Schroeder *et al.*, which unmistakably indicate a large probability (92 per cent.) for the occurrence of the sequence gly-pro suggest that they in fact occur in this position.

As mentioned earlier, if we assume that a hypro residue occurs in every position where it can form a strong linkage through a hydrogen bond with a neighbouring rod, then the percentage of hypro residues comes to be 11 per cent. It is indeed gratifying to note that the maximum percentage of hypro residues observed in mammalian collagen (Gustavson, 1954) is 10·9.*

(ii) *Infra-red absorption.*—It has been shown already (Ramachandran, 1954) that in a simple 3–10 helix, the directions of the NH and CO vibrations are in broad agreement with infra-red data. In Table II below are listed the angles made by these directions for the three types of residues in the coiled coil structure. It will be seen that the expected dichroism is again in good agreement with observation.

(iii) *X-ray pattern.*—It is found that the structure agrees fairly well with the observed X-ray pattern. The equatorial transform has been published in the preliminary report (Ramachandran and Kartha, 1955) and is not included in this paper. It shows a large peak at the origin which drops

* We are grateful to Dr. Gustavson for pointing out the correct value in a personal communication to G.N.R. This value seems to be in even better agreement with the structure than the value 10 per cent. mentioned in the preliminary report. However, it must be stated that this exact agreement must be considered somewhat fortuitous, as the theoretical value will change if the parameters in the structure are altered even slightly.

TABLE II

Orientation of NH and CO bonds in the coiled coil structure

Residue type	Angle made with axis of major helix by	
	NH bond	CO bond
1	75°	74°
2	88°	86°
3	80°	76°

to zero corresponding to a spacing of 7·4 A. There is a second peak at 4·7 A and a third one at 2·2 A. Thus, the occurrence of the first and second order reflections of the (100) planes in dry collagen and the absence of the 110 reflections are explained. Calculations (made by Y. T. Thathachari) show that the minimum at 7·4 A shifts to longer spacings with increasing water content, thus explaining the continued absence of the 110 reflections. The occurrence of a diffuse spot at 4·4 A is to be attributed to the second peak in the Fourier transform along the equator. So also, the cylindrical Patterson diagram of collagen, calculated by Yakel and Schatz (1955), is in good agreement with the backbone of the structure, as described in the earlier report.

The optical transform of the structure reported in *Nature* was obtained by Dr. Cowan and Mr. North of King's College, London and is shown in Fig. 6 *a*, Plate IV, along with the X-ray diffraction pattern obtained by Mr. Ambady of this laboratory with the fibre inclined at an angle of 75° to the X-ray beam (Fig. 6 *b*). The remarkable similarity of the two patterns is indicative of the fact that the structure is essentially correct. It must be mentioned that the optical transform was obtained with measurements taken on the projection of the structure, so that it is not very accurate. The following features of optical transform may be noted:—

(*a*) The prominent layer lines are third, seventh and tenth.

(*b*) The maximum intensity in the fourth layer line occurs at about $\xi = 0{\cdot}20$, as found by experiment.

(*c*) Belts of strong intensity occur near about the row lines corresponding to $\xi = 0{\cdot}35$ and $0{\cdot}7$, again as is found in the X-ray pattern.

In the X-ray pattern yielded by wet collagen fibres, the diffraction pattern is spread out along the layer lines, so that the intensity distribution in this

pattern is a much better representation of the Fourier transform of the collagen protofibril than the pattern given by dry specimens. All the features (*a*), (*b*), (*c*) are exhibited by the pattern of wet collagen also (Ramachandran and Ambady, to be published).

(iv) *Electron micrographs.*—The existence of a long-spacing of 618 A along the fibre axis has been shown to follow from the condition that the protofibrils are packed in a hexagonal array. If the arrangement is exactly hexagonal, it also follows that the distribution of side-groups will repeat six times within this spacing, although the three chains make a complete number of revolutions, (namely 7) only in 618 A. Such an approximately equal six-fold subdivision has been observed in stained specimens by Schmitt and Gross (1948), and also by others. Actually, the subdivision is not exact. This would happen if the lattice is not truly hexagonal, but monoclinic, as is found to be the case with native fibres (North, Cowan and Randall, 1954). However, a full discussion of this feature appears to be premature at this stage of our understanding of the collagen structure.

So also, the existence of longer spacings of the order of 2000 A may be explained on the basis of a different scheme of fitting the helix of the protofibril in a hexagonal lattice, but this suggestion is purely tentative.

(v) *Shrinkage temperature and gelatin.*—It has been reported that the shrinkage temperature of collagen from different sources is directly correlated to the hydroxyproline content (Gustavson, 1954, 1955). This fact is readily explicable on the basis of the present structure, in which the important cross-links between different protofibrils are produced by the hydrogen bonds occurring between hydroxyproline OH groups and the carbonyl oxygen atoms. Thus, the greater the number of such linkages, the higher should be the shrinkage temperature.

It is suggested that in thermally shrunk collagen, the protofibrils remain intact, although they may take a folded configuration. Consequently, one would expect to find the X-ray pattern to be a highly disoriented representation of the pattern in the normal state. This is indeed found to be the case, for the pattern contains a fairly sharp ring corresponding to 2·86 A in addition to the diffuse ring at about 4·4 A. Also, it follows that an oriented arrangement could be recovered by cooling and stretching the specimens.

In the diffraction pattern of gelatin (Hermann, Gerngross and Abitz, 1930), the 2·86 A ring is comparatively weak, but the rest of the pattern is practically the same as for thermally shrunk collagen. This again shows that the protofibrillar structure is practically undisturbed in gelatin, but that the fibrils are highly distorted and folded in gelatin. The occurrence

of a ring corresponding to 12 to 17 A in gelatin shows that even in this state, there is an approximately parallel aggregation of the cylindrical rods at the protofibrillar level. Excepting this ring, the diffraction pattern of gelatin should be considered as the spherical transform of the collagen protofibril.

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SUMMARY

The paper reports the details of the revised structure of collagen. It is composed of three helical polypeptide chains, each of which has ten residues in three turns of a left-handed helix. The three chains are further wound into a superhelix in the form of intertwined coiled coils. The major helix is right-handed and makes one turn in thirty residues. The structure has reasonable hydrogen bonds, two for every three residues. The three-chain cylindrical rods are stacked together in hexagonal array and stabilised by cross-linkages through hydroxyproline side-groups. In trying to fit these in the lattice, a slight uncoiling of the major helix is required, resulting in a repeat of 618 A along the fibre axis. The proposed structure is in good agreement with the infra-red and X-ray data and also fits in broadly with the amino-acid composition and other properties of collagen.

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(b)

(a)

FIG. 6. (a) Optical transform of the structure.
(b) Diffraction pattern of stretched fibre kept at 75° to the X-ray beam. Note the almost exact correspondence in the central region of the top halves of the two figures.

THE PROPAGATION OF LIGHT IN ABSORBING BIAXIAL CRYSTALS

II. Experimental

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§ 1. Introduction

The mineral iolite available in South India shows a very pronounced pleochroism: certain specimens of this material appear practically colourless and transparent in certain orientations, but show an intense blue colour in certain others. Accordingly, a plate cut normal to one of the two optic axes of this orthorhombic mineral was found very suitable for qualitatively verifying some of the rather unexpected results regarding the properties of the singular axes in absorbing crystals that had been derived in the theoretical treatment of Part I. As the plate also exhibited in a striking manner the other well-known phenomena characteristic of absorbing biaxial crystals, it was thought worthwhile to publish illustrations of these phenomena (together with a qualitative verification of some of the more detailed aspects of the theory that had hitherto not been convincingly demonstrated). Broadly speaking, sections 2–4 do not cover original ground, and the phenomena they deal with are also explained, for example, in Pockels' *Lehrbuch*.[1]

In Part I of this paper[2] it was shown that the optical behaviour of absorbing crystals not possessing optical activity could be regarded as due to the effects of linear birefringence and linear dichroism superposed continuously along the depth of the medium.* The peculiar features in biaxial crystals arise because here the principal planes for the usual operation of birefringence do not coincide with those for the operation of dichroism. In spite of the simplification which such a method of consideration represents over the more rigorous electromagnetic theory, the features of propagation in an absorbing biaxial medium are still somewhat complex. Therefore, in explaining the experimental phenomena involved we shall start by assuming the elementary theory of Mallard; and we shall broadly indicate in §§ 2–4, how the experimental results themselves point out the need for a more refined

* See Note at the end of the paper for references to some earlier work.[3]

theory, as well as to some of the results of such a theory. (The theoretical treatment in many standard texts[4–7] finally amount in practice to the assumptions of Mallard's theory—except for a slight change in the form of the absorption ellipsoid which may be neglected for directions near an optic axis.)

The photographs (Figs. 2–15) illustrated in this paper were all taken with sodium light illumination in convergent light; the simplest method of observing the phenomena which they reproduce is by holding the plate close to the eye and looking towards an extended source of light through the plate —there being arrangements for introducing auxiliary appliances (such as polaroids and quarter-wave plates) both in front of and behind the crystal. Each point in the convergent light figure obviously corresponds to a definite direction of propagation: to visualise this we may represent all directions as passing through the centre of a sphere and defined by their intersection with the spherical surface. The region surrounding an optic axis may then be approximated by a plane (the plane of the paper in Fig. 1), the central point O in the convergent light figure corresponding to the optic axis, and the horizontal line $X_k X_k'$ to the axial plane. (Since the axial angle is not small, the second optic axis does not appear in the figure.)

§ 2. PROPERTY OF THE OPTIC AXES IN ABSORBING CRYSTALS

When a plate of an absorbing biaxial crystal cut normal to an optic axis is examined in convergent light between crossed polaroids, the optic axial direction does not in general appear extinguished (as in transparent crystals) but shows two extinction positions as the crossed polaroids are rotated round together—its behaviour in this respect being similar to that of a *non*-axial direction in a transparent specimen. In the case of an orthorhombic crystal, these extinction positions occur when the vibration-direction of the polariser lies either along or perpendicular to the axial plane; the appearance of the figure which is the same in both cases, is given in Fig. 2, the dark isogyre lying along the axial plane as in transparent crystals. On the other hand Fig. 3 shows that for an intermediate position where the vibration-directions of the crossed polaroids make angles of 45° with the axial plane, the optic axial direction is not extinguished by a vertical isogyre.

Along the optic axis, therefore, two linearly polarised vibrations are propagated. If we take the elliptic section of the absorption ellipsoid normal to the optic axis, these two waves are linearly polarised along the principal radii of this section—which lie parallel to OX_k and OY_k in the case of the orthorhombic crystal iolite. The characteristic feature distinguishing these two waves is not any difference in their refractive indices, but the fact that they have different coefficients of extinction k_1 and k_2, where $1/\sqrt{k_1}$ and

$1/\sqrt{k_2}$ are the principal radii of the absorption ellipsoid lying along OX_k and OY_k respectively; the absorption for the vertical vibration-direction is much greater than that for the horizontal vibration-direction in the case of iolite.

The existence of dichroism along the optic axial direction is sometimes[4, 5] expressed by the statement that the absorption of a wave travelling along an optic axis depends on its plane of polarisation. This statement is however somewhat misleading for if a wave incident in the direction of an optic axis has its vibration-direction inclined at an arbitrary angle to the axial plane, the disturbance is not propagated with a single specific coefficient of absorption; on the other hand, it is split up into two waves with different coefficients of absorption polarised along and perpendicular to the axial plane, and the vibration-direction of the wave emerging from the plate will not be the same as that of the incident wave but would have turned towards the less absorbed component—towards the axial plane in the case of iolite. This is the reason why the optic axial direction does not in general appear extinguished between crossed polaroids. According to this explanation the optic axial direction can be extinguished by rotating the analyser away from the crossed position, and this has been done in Fig. 4. The extinction would not have been complete if there had been any (sensible) difference in the refractive indices of the waves.

An estimate of the dichroism along the axial direction will be useful later, and this can be made if, in Fig. 4, the inclinations ψ and ψ' that the incident and emergent vibrations, make with the axial plane are known. These angles were estimated to be roughly $(90° - 12\frac{1}{2}°)$ and $12\frac{1}{2}°$ respectively. From equation (1) of Part I we then get $kz = 3$ approximately, where k is the difference in the coefficients of absorption and z the thickness.

[The azimuths of the polariser and analyser had not been directly noted during the experiment. Hence ψ and ψ' were indirectly determined by noting that the two diameters along which the ring system is absent in Fig. 4, are inclined at roughly 25° to the axial plane. We then use the fact that these diameters represent directions where one of the principal planes of refraction coincide with the vibration-direction either of the polariser or of the analyser. (Since the diameters where this occurs are not highly inclined to the axial plane, the waves here may be regarded as linearly polarised.)]

§ 3. Brewster's Brushes

With neither polariser or analyser introduced, the convergent light figure reveals the two dark Brewster's absorption brushes which appear in the plane perpendicular to the axial plane (Fig. 5).

The broad features of this phenomena are explicable on Mallard's theory. In Fig. 1 in the text, each double-headed arrow at the border gives the vibration-direction of the faster wave for all points on the corresponding radius

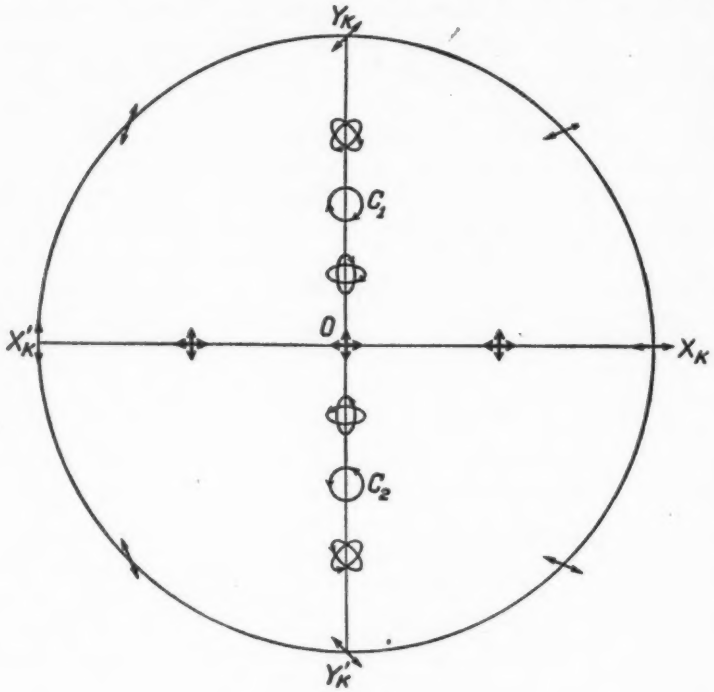

FIG. 1

X_k, X_k' = axial plane; O = optic axis; C_1, C_2 = singular axes.

of the field of view (assuming Mallard's theory). Now the absorption coefficients for the two vibrations propagated in any direction are determined by the intercepts that these vibrations make on an absorption ellipsoid; and though the orientations of these vibrations remain constant along any one diameter, they turn round rapidly with the diameter—giving rise to the Brewster's brushes.

The elliptical section of the absorption ellipsoid made by the plane of the paper in Fig. 1, may also be taken as the section normal to any direction of propagation in the small angular range covered by the convergent light figure. The mean of the two absorption coefficients may then be considered a constant for all directions in the angular range under consideration.

(On Mallard's theory this follows from a property of any two perpendicular radii of an ellipse, and in the more exact theory by the equations 15 of Part I.) Under this condition it may be easily shown that the sum of the intensities of the two waves emerging from the plate will be *minimum* along those directions where the two waves (into which the incident unpolarised light is split) are absorbed equally strongly; and that the sum of the intensities of these waves will be *maximum* along directions where the two waves differ in their absorption coefficients to the maximum extent.

Now the dichroism will be greatest where the waves are linearly polarised along the principal planes of absorption OX_k and OY_k—a situation which occurs for directions along the axial plane in the case of an orthorhombic crystal like iolite. For directions in the perpendicular plane Y_kY_k' the principal planes of refraction are inclined at 45° to the principal planes of absorption; hence the dichroism should be zero for all points on Y_kY_k' (the optic axial direction O being excepted in the case of Mallard's theory, the small strip C_1C_2 being excepted according to the exact theory). This explains the occurrence of the Brewster's brushes in the plane perpendicular to the axial plane.

§ 4. Phenomena Not Explicable on the Elementary Theory

(*a*) *The elliptical polarisation of the waves.*—Referring to Fig. 3, we note that when the plate is viewed between crossed polaroids set in the diagonal position it is not the optic axial direction *alone* that remains unextinguished; the region of non-extinction extends over a finite vertical strip passing through the optic axis, and, in fact, the extinction along the vertical ' isogyre ' becomes perfect only at the boundary of the figure.

An important inference may be drawn from this phenomenon. Along the optic axial direction (where the birefringence is zero) the two waves propagated are linearly polarised along the principal planes of absorption (*i.e.*, along the principal radii of the normal section of the absorption ellipsoid); for directions appreciably inclined to the optic axis (where the birefringence becomes notable) the waves may be regarded as linearly polarised along the principal planes of refraction. But the transition between these two extreme stages (*i.e.*, from waves polarised along the principal planes of absorption to waves polarised along the principal planes of refraction) occurs in some continuous fashion—not discontinuously, as soon as the optic axial direction is departed from, as is required by Mallard's theory. This is also to be expected on the simple ground that the phenomena that are physically observable along the optic axial direction are not really explained by postulating a discontinuous property for the exact axial direction, since we are

always concerned with pencils of finite (even if small) convergence. For directions along the axial plane the principal planes of refraction and absorption coincide so that the horizontal isogyre in Fig. 2 is dark and continuous.

Further, those points in the immediate vicinity of the optic axis O, which lie along the vertical line $Y_k Y_k'$, cannot be extinguished even on rotating the crossed polaroids together, away from the diagonal setting—which means that the waves here must be *elliptically* polarised. This property resembles that of transparent optically active birefringent crystals where two extinction positions cannot be obtained between crossed polaroids.

(*b*) *Phenomena with polariser or analyser alone.*—For any direction along the axial plane the two waves are polarised along and perpendicular to the axial plane; the two waves have respectively the least and greatest absorption coefficients of all the waves travelling along any direction in the field of view. Hence if a polariser be set with its vibration-direction perpendicular to the axial plane, a dark brush appears along the axial plane, as in Fig. 9; while if it is set along the axial plane, a white brush appears in the same position as in Fig. 6. These two photographs incidentally demonstrate the existence of pleochroism along the axial direction.

The most interesting feature about Figs. 6 and 9, is, however, the occurrence of the idiophanic interference rings, which appear conspicuous in the plane perpendicular to the axial plane. As the polariser is turned from its position along the axial plane in Fig. 6 to that perpendicular to the axial plane in Fig. 9, the figure changes in a manner which appears to depend on the absorption of the specimen. In the present case, the diameter along which the rings are most conspicuous first turns with the polariser (Fig. 7); next a dark brush gets detached from this diameter and turns round towards the axial plane, while the ring system again turns back towards a position perpendicular to the axial plane (Fig. 8).

The occurrence of idiophanic interference rings cannot be expected if the states of polarisation propagated along any general direction are two orthogonal linear vibrations (as assumed in Mallard's theory), or alternatively two orthogonal elliptic vibrations—as occurs in transparent optically active crystals. For even when two such beams are completely coherent (a condition automatically ensured by polarising the incident light), they will be incapable of interference with one another—unless brought to the same state of vibration by the use of a suitable analyser. The two vibrations propagated along any general direction may therefore be assumed to be two *non-orthogonal* elliptic vibrations, propagated with different velocities and absorption coefficients. (Two elliptic vibrations having their major axes

crossed and their ellipticities equal, and which are described in opposite senses are said to be orthogonal; any two vibrations not satisfying this condition are non-orthogonal.)

Figure 10 shows the photograph obtained by using an analyser alone, set in the same position as the polariser in Fig. 8, the exposures in the two cases being identical. The two photographs appear identical. It is possible to deduce from this that the non-orthogonal vibrations propagated along any general direction must be of the form of two similarly rotating elliptic vibrations having their major axes crossed and their ellipticities equal. If the non-orthogonal vibrations were of other forms (as for example, two linear vibrations inclined at an angle different from 90°) the observed identity of the effects obtained with a polariser alone and an analyser alone could not, in general, be expected.†

Along the axial plane X_kX_k', the principal planes of refraction and absorption are coincident, so that the vibrations for directions of propagation in this plane are linear and orthogonal, as indicated in Fig. 1. This explains the absence of the idiophanic rings along the axial plane in Figs. 6–9.

As we proceed outwards from the optic axis along the radius OY_k, the change in the states of polarisation of the two waves may be easily followed on the Poincaré sphere by applying the principle of superposition developed in Part I. For these directions the principal planes of refraction and absorption have their maximum mutual inclination of 45°, and effects connected with the ellipticity may be expected to be most pronounced. Referring to Fig. 3 in Part I of this paper, as the linear birefringence increases from zero, the two states of polarisation (initially coincident with the principal planes of absorption X_k and Y_k respectively) will move downwards along the arcs X_kC_r and Y_kC_r respectively till both states become coincident with the pole C_r when the linear birefringence δ becomes equal to the linear dichroism k; as the birefringence further increases, the two states again become distinct and move upwards along the meridians of longitude C_rX_r and C_rY_r respectively.

Physically this means that as we proceed outwards along the radius OY_k, the two vibrations (initially polarised along the principal planes of absorption) open out into two right elliptic vibrations which become two identical circular vibrations at C_1; further on these split up into two elliptic vibrations with major axes along the principal planes of refraction, these elliptic vibrations tending to the form of two orthogonal linear vibrations at the

† This will be proved in a later paper dealing with absorbing crystals which are also optically active, where the effects obtained with polariser and analyser are different.

border of the field of view. For directions on the other side of the axial plane the vibrations are left-elliptic. The velocities of the waves are equal for all directions lying within the small vertical strip C_1C_2 (as may be seen by setting $2\phi = \pi/2$ in Equation 14, Part I). But for all other directions in the vertical plane Y_kY_k', the absorption coefficients of the waves are equal, the velocities being, in general, different (as may be seen by setting $2\psi = \pi/2$ in Equation 15, Part I).

For any given angular distance from the axial direction, the ellipticity of the waves (and hence their departure from orthogonality) is maximum for directions lying in the vertical plane Y_kY_k'. Consequently the idiophanic rings may be expected to be most prominently seen, if the polariser is adjusted such that the ring system lies perpendicular to the axial plane as in Figs. 6 and 9. (This effect does not appear to be very pronounced.) The considerations of the previous paragraph show that the rings will appear in the vertical plane when the polariser is set along or perpendicular to the axial plane; for the two waves propagated along any direction in the vertical plane Y_kY_k' (excluding the small strip C_1C_2) will then have equal amplitudes and will consequently be capable of showing the maximum interference effects. (The result of this paragraph has been proved by means of a more exact mathematical treatment in Pockels.)

(*c*) *Interference effects in Brewster's brushes.*—Figure 5 shows distinct traces of periodic maxima and minima in the Brewster's brushes, in the region near the optic axis. The occurrence of such interference effects may be broadly expected on the following grounds. When unpolarised light is split into two orthogonally polarised vibrations, these vibrations will be completely incoherent; but when the incident unpolarised light is being split into two *non-orthogonally* polarised vibrations, as in the present case, the two vibrations must necessarily be considered as partially coherent—for if they were incoherent it can be shown that the resultant beam would be partially polarised and not unpolarised (see *e.g.*, reference 8). Since the beams are partially coherent (to an extent which depends on their departure from orthogonality), they will be capable of restricted interference with one another (to an extent which depends again on their departure from orthogonality). The interference effects are therefore very feeble, being noticeable only in the immediate vicinity of the singular axes—unlike the idiophanic rings which are fairly prominent because complete coherence of the beams is ensured by polarising the incident light. It can be shown‡ that the maxima

‡ This will be shown incidentally in a later paper dealing with absorbing crystals possessing optical activity.

and minima in the Brewster's brushes must occur in the same position as those in the idiophanic rings obtained by setting the polariser along the axial plane, and this is verified by comparison with Fig. 6.

The explanation of the Brewster's brushes given in §2 is however still applicable. For though the intensity emerging along any direction is not merely equal to the sum of the intensities of the two waves emerging in that direction (because of an additional term expressing their interference), it is this sum that broadly locates the position of the brushes.

From the standpoint of the method of superposition we may also regard the interference effects as arising because the crystal itself acts both as polariser and analyser—and a similar explanation could also be given for the idiophanic rings.

§ 5. The Singular Axes

(*a*) *Examination between circular polariser and crossed circular analyser.*—The point C_1 represents a direction where only a right-circular vibration can be propagated unchanged; while the point C_2 represents a direction where only a left-circular vibration can be propagated without change of form. These two directions are termed the Windungsachen or singular axes. We shall now give a direct confirmation of the existence of these axes; the only previous experimental work dealing directly with the property of these axes was, as we shall see, not interpreted in a correct manner.

In front of the crystal plate is kept a ' left-circular polariser ' (*i.e.*, an arrangement which transmits left-circularly polarised light when unpolarised light is incident on it). Behind the crystal is kept a ' right-circular analyser ' (*i.e.*, an arrangement which completely cuts off left-circularly polarised light). Under such conditions a transparent crystal will show a system of circular rings with a black spot at the optic axis, where the incident circularly polarised light can be propagated unchanged. In the present case the dark spot occurs slightly to one side of the optic axis (Fig. 11), corresponding to the direction of the lower singular axis C_2. (This is seen better in Fig. 16, which was taken with another plate of iolite having less total absorption.) When the crystal plate is viewed between a right-circular polariser and a crossed circular analyser, the other singular axis C_1 appears extinguished (Fig. 12). The angle between the singular axes is seen to be very small, and has been exaggerated in Fig. 1.

(A quarter-wave plate preceded by a polaroid at 45° to the principal planes, together constitute a convenient circular polariser; a quarter-wave plate followed by a polaroid at 45° to the principal planes, similarly constitute a circular analyser.)

In Part I, Section 4 *c*, it was shown that the vibrations propagated in any direction are right- or left-elliptic according as the principal plane of refraction OX_r (corresponding to the slower wave, in the absence of absorption) makes a negative or positive acute angle with the principal plane of absorption OX_k (corresponding to the less absorbed component in the absence of birefringence). Accordingly in Fig. 1, the sense of description of the elliptic vibrations are correct only if we assume that the arrows at the border give the *faster* vibration-direction (for all points on the corresponding radius) in the absence of absorption. That this assumption was indeed correct, was verified by using the fact that at the border of the field of view the refractive indices and states of polarisation are substantially the same as in the absence of absorption. The polariser and analyser were set along and perpendicular to the axial plane respectively, as in Fig. 2, and a quarter-wave plate was introduced immediately behind the crystal plate with its slow vibration-direction making an angle of $+ 45°$ with respect to OX_k. The rings along OY_k moved outwards indicating a diminution of the phase retardation for such directions, while those appearing on the line OY_k' moved towards the centre. This confirmed that the right- and left-circular singular axes occur respectively on the sides of the axial plane that are to be expected according to theory.

§ 6. EFFECTS WITH INCIDENT CIRCULARLY POLARISED LIGHT

When the incident light is circularly polarised it is propagated without change of form along one of the singular axes. In Part I it was shown that for the direction corresponding to the other singular axis, the emergent intensity will not be either zero or negligible (as had been thought by Voigt[1]), but should in fact be greater than the intensity emerging in the direction of the first singular axis. This was confirmed in the following manner. When examined between a left-circular polariser and a crossed circular analyser, we have seen that the singular axis C_2 where the incident vibration is propagated without change of form appears as a dark spot (Fig. 11). If now the circular analyser is removed, the same singular axis appears darker than the other singular axis (which appears within a bright spot), as may be seen in Fig. 13.

Similarly Fig. 14 shows the appearance presented when the incident light is right-circularly polarised. It will be seen by comparison with Fig. 12, that the upper singular axis C_1 where the incident vibration is propagated without change of form appears the darker of the two. Along the other singular axis C_2 the state of vibration alters progressively along the depth of the medium, according to the theoretical analysis of Part I. The emergent

vibration in this direction will be elliptically polarised, the major axis of the vibration lying along OX_k (which is the principal plane corresponding to the less absorbed component). Hence if a quarter-wave plate is introduced behind the crystal plate with its principal planes along OX_k and OY_k, the elliptic vibration emerging in the direction of the lower singular axis C_2 will be reduced to a linear vibration at an angle θ to the axial plane—where $\tan\theta$ is the ratio of the minor to the major axis of the elliptic vibration. Thus by introducing a polaroid behind the quarter-wave plate it should be possible to extinguish the singular axis C_2. To verify this, the point C_2 was marked with an ink spot on the ground glass of the camera, after having determined its exact location by the position of the black spot in the arrangement of Fig. 11. By rotating the polaroid it was found that a point of extinction lying on the vertical line Y_kY_k' could be taken continuously *through* the singular axis C_2 (see next paragraph). Figure 15 shows the photograph taken when C_2 has been extinguished in this manner. The angle θ was roughly estimated at 25°; this is roughly the magnitude to be expected since from Equation 18 of Part I we should have: $\tan(\pi/4 + \theta) = kz$, where kz has already been estimated to have a value near 3 (refer § 2).

More generally (with the incident vibration right circularly polarized, as before) the elliptic vibration emerging along any direction on the vertical plane Y_kY_k' will have its principal axes along OX_k and OY_k. This is a consequence of the fact that for any such direction the principal planes of refraction and absorption are inclined at 45° to one another—the corresponding diameters X_rY_r and X_kY_k on the Poincaré sphere being at right angles to one another as in Fig. 3 of Part I; in that figure, if C_r represents the incident vibration, the movements ds_r and ds_k will always lie along the same line-element, so that the emergent vibration will necessarily be represented by some point on the arc $C_rX_kC_lC_r$. Hence if a quarter-wave plate is introduced with its principal planes along OX_k and OY_k (as described in the last paragraph) the vibrations emerging along all the directions in the vertical plane Y_kY_k' will be reduced to linear vibrations (with various azimuths of polarisation). The polaroid behind this quarter-wave plate was set at 45° to the principal planes in such a position that the upper singular axis C_1 where the incident vibration is propagated unchanged appeared extinguished. By rotating the polaroid away from this position in the proper direction, the point of extinction could be moved continuously downwards *through* the lower singular axis C_2.

§ 7. EXAMINATION BETWEEN ELLIPTIC POLARISER AND CROSSED ELLIPTIC ANALYSER

It would be desirable to confirm that the principal axes of the two elliptic vibrations propagated without change of form along any direction, do not (in general) coincide with the principal planes of refraction—for even the behaviour of the idiophanic rings can be sufficiently well explained without taking this factor into account (see Pockels). We shall describe an experiment confirming this for directions represented by the points on C_1C_2, where the principal axes of the elliptic vibrations should lie parallel to OX_k and OY_k whereas the principal planes of refraction are inclined at 45° to these directions.

The arrangement used was that described by Ramachandran and Chandrasekharan[9] in another connection. The crystal was kept between crossed polaroids which could be rotated around together. A quarter-wave plate was set immediately behind the first polaroid, with its principal planes along OX_k and OY_k; another quarter-wave plate was set immediately in front of the second polaroid with its principal planes turned by 90° with respect to the first quarter-wave plate. If the crystal plate were absent, then obviously the elliptically polarised light produced by the arrangement in front would be automatically cut out completely by the arrangement behind, independent of the setting of the ganged polaroids. In the presence of the crystal plate, any direction where the incident elliptic vibration is propagated without change of form will appear extinguished. (The principal axes of the incident elliptic vibration will always lie along OX_k and OY_k.)

Starting with the polariser set along the axial plane, the optic axial direction O appears extinguished as in Fig. 2. As the ganged polaroids are rotated away from this setting in the proper direction, the point of extinction on the line Y_kY_k' moves upwards from O till it reaches the singular axis C_1 (polaroid at 45° to principal planes of $\lambda/4$ plate). As the ganged polaroids are rotated still further the point of extinction retraces its path, moving from C_1 back to O (polariser set perpendicular to axial plane). Further rotation through 90° causes the extinction to move upto C_2 and back to O.

The above experiment provides a direct confirmation of the theoretical result that the two elliptic vibrations propagated along these directions have their major axes crossed, have the same ellipticity and are described in the *same* sense. It also shows that the two elliptic vibrations gradually degenerate into two (identical) circular vibrations as the singular axis is approached.

The author is very grateful to Prof. Sir C. V. Raman for his keen interest in this investigation.

§ 8. Summary

The phenomena shown in convergent light by a section plate of iolite cut normal to an optic axis are discussed and illustrated. Of particular interest among the photographs reproduced are those taken (*a*) between circular polariser and crossed circular analyser, demonstrating the existence of the two singular axes (a right-circular vibration alone being propagated unchanged along one singular axis, and a left-circular vibration alone along the other); (*b*) with a circular polariser alone, confirming the theoretical prediction in Part I that the singular axis where the incident circular vibration can be propagated unchanged should appear darker than the other; the state of the elliptic vibration emerging from the brighter singular axis was also qualitatively in accord with theory. Examination between elliptic polariser and crossed elliptic analyser demonstrated that along any general direction in an (optically inactive) absorbing biaxial crystal, two similarly rotating elliptic vibrations can be propagated unchanged—the major axes being crossed but not coincident with the principal planes of refraction.

§ 9. References

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§ 10. Descriptive Notes on the Plates

All photographs were recorded with a plate of iolite cut normal to an optic axis, the plate being held normally with the axial plane horizontal.

Phenomena with polariser and analyser

Fig. 2. Crossed polaroids: vibration directions along and perpendicular to axial plane. (Optic axis extinguished.)

Fig. 3. Crossed polaroids: vibration directions at 45° to axial plane. (Optic axis not extinguished.)

Fig. 4. Non-crossed polaroids: vibration directions equally inclined to axial plane. (Optic axis extinguished.)

Brewster's brushes and idiophanic rings

FIG. 5. Brewster's brushes, with neither polariser nor analyser. (Traces of interference rings seen.)

FIGS. 6, 7, 8 & 9. Idiophanic rings with polariser *alone* at various settings: along the axial plane in Fig. 6, perpendicular to the axial plane in Fig. 9, and at two intermediate orientations in Figs. 7 and 8.

FIG. 10. Idiophanic rings with analyser alone, set in the same position as the polariser in Fig. 8.

Properties of the singular axes

FIG. 11. Left-circular polariser and right-circular analyser. (Lower singular axis extinguished.)

FIG. 13. Left-circular polariser alone. (Lower singular axis appears darker than the other.)

FIG. 12. Right-circular polariser and left-circular analyser. (Upper singular axis extinguished.)

FIG. 14. Right-circular polariser alone. (Upper singular axis appears darker than the other.)

FIG. 15. Right-circular polariser and an elliptic analyser adjusted to cross out the elliptic vibration emerging from lower singular axis.

FIG. 16. Circular polariser and crossed circular analyser. (Photograph taken with a more lightly coloured plate: lower singular axis extinguished.)

NOTE

In Part I of this paper it was shown that the features of wave-propagation in absorbing biaxial crystals may be regarded as due to the effects of linear birefringence and linear dichroism superposed continuously along the depth of the material; this idea was worked out by a simple geometric method using the Poincaré sphere (as also by a more lengthy algebraic method). A series of papers published earlier by Clark Jones[3] dealing with 'A new calculus for the treatment of optical systems' has since then come to the notice of the author; the comprehensive calculus elaborated in the first half of that series uses the representation of any optical device by 2×2 complex matrix (operating on the electric vector of the incident light). In Part IV of that series one of the applications that has been made of the calculus is the derivation of the matrix for a plate of an absorbing crystal by a method which is physically equivalent to the method of superposition; the matrix corresponding to an infinitesimal path has been considered in Part VII.

FIG. 2

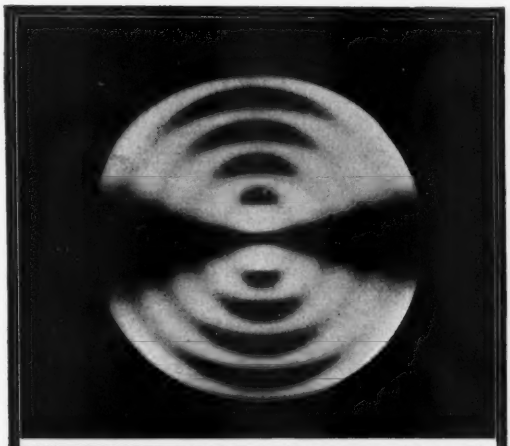


FIG. 3

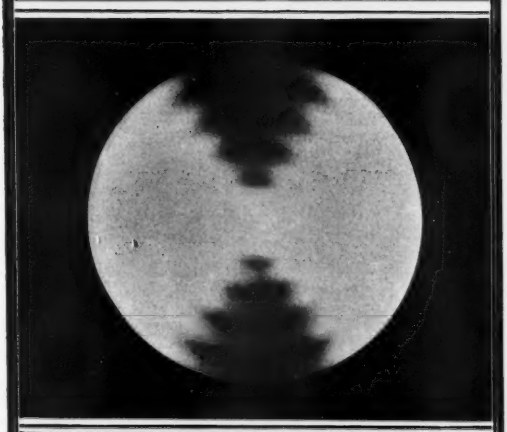
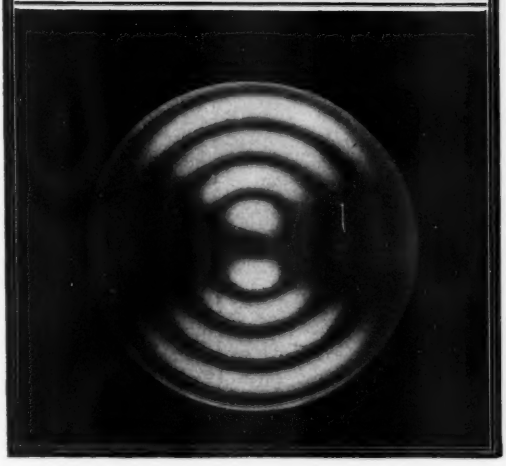


FIG. 4



Phenomena with polariser and analyser

FIG. 5

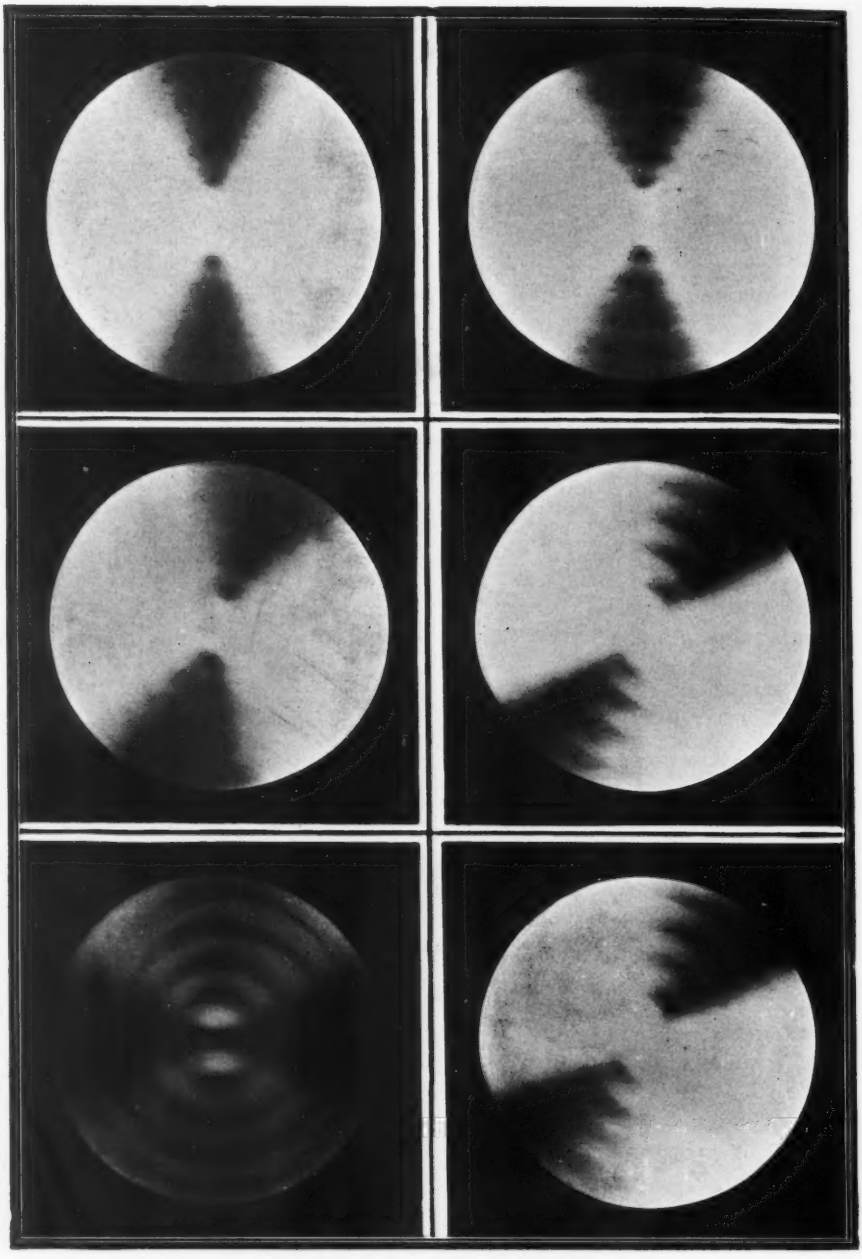


FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIG. 10

Brewster's brushes and idiophanic rings

FIG. 11

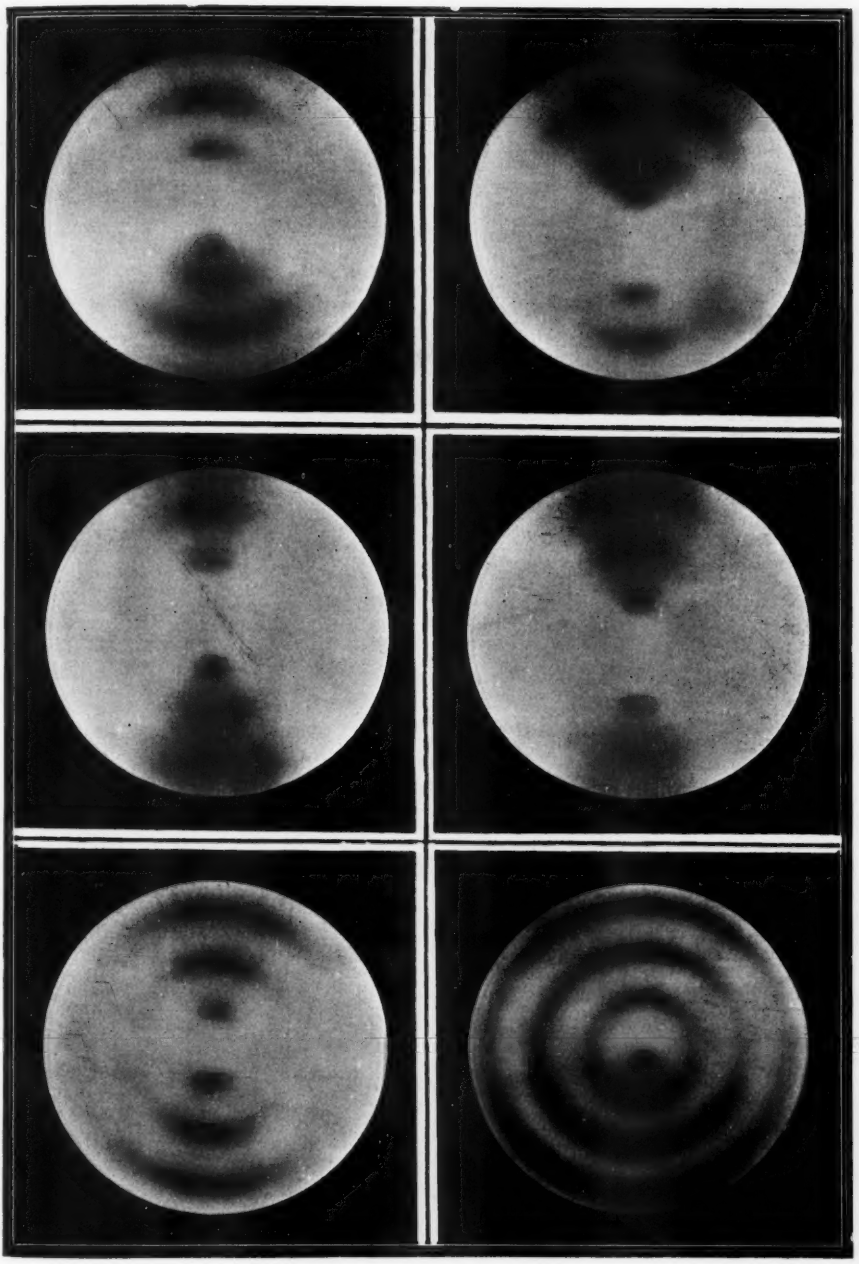


FIG. 12

FIG. 13

FIG. 14

FIG. 15

FIG. 16

Properties of the singular axes

SEARCH FOR PHYSIOLOGICALLY ACTIVE COMPOUNDS

Part 1. Synthesis of 7-Amino and 7-Halo-4-methyl-3-phenyl Coumarins

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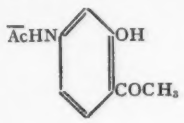
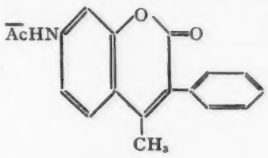
(Communicated by Prof. T. R. Seshadri, F.A.SC.)

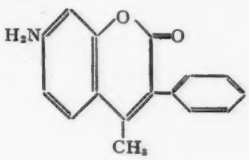
7-Hydroxy-4-methyl-3-phenyl coumarins and their methyl ethers were reported by Seshadri and Varadarajan[1] to be highly toxic to fish. The toxicity of these compounds was explained on the basis of their close resemblance to isoflavones. Recently Naito and others[2] reported 7-amino-4-methyl coumarin to be active against tubercle bacilli (1 : 64,000). It was therefore considered desirable to prepare 7-amino and 7-halo-4-methyl-3-phenyl coumarins which may possess better bactericidal and insecticidal properties. These compounds have not been prepared previously. The method of Bargellini[3] for the preparation of 3-phenyl-4-methyl umbelliferones was improved by Seshadri and Varadarajan.[4] It does not seem to have been applied for the preparation of 7-substituted coumarins other than the 7-hydroxy derivatives.

In the present case, *p*-acetamino-*o*-hydroxy acetophenone[5] (I) obtained by the Fries migration of *m*-acetamino phenyl acetate, was condensed with sodium phenyl acetate and acetic anhydride. The resulting product on hydrolysis and recrystallisation from hot alcohol gave 7-amino-4-methyl-3-phenyl coumarin (II). From it the corresponding 7-halogen substituted

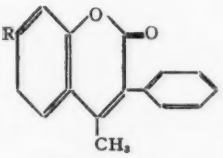


(I)





(II)



$R = \begin{Bmatrix} Cl \\ Br \\ I \end{Bmatrix}$

(III)

coumarins (III) were prepared through diazotisation and Sandmeyer reactions. This indirect method was adopted for the preparation of the halogen compounds, as direct halogenation[6–9] usually results in the 3, 6 and 8 positions being substituted in the order of availability, and other synthetic routes like the Perkin and Pechmann condensations involve preparation of difficultly accessible intermediates and generally result in poor yields.

The 7-amino compound was feebly toxic to fish whereas the 7-halogen derivatives were found to possess appreciable toxicity. Comparison of their toxicity to fish with the corresponding 7-hydroxy compound showed that the halo-compounds were far more toxic. Full details of the physiological activity of the compounds towards fish and bacteria will be reported later.

EXPERIMENTAL

(i) *7-Acetamino-4-methyl-3-phenyl coumarin*

p-Acetamino-*o*-hydroxy-acetophenone[5] (4 g.), sodium phenyl acetate (8 g.) and acetic anhydride (50 ml.) were heated at 120° C. for ½ hr. and then at 180° C. for 4 hrs. The reaction mixture was poured in water and allowed to stand overnight when a pasty mass separated. The water was decanted off and the pasty mass was treated with cold alcohol when the solid acetate separated. It was filtered, washed with cold alcohol, dried (2·5 g.) and recrystallised from glacial acetic acid; white glistening plates, m.p. 266° C. (Found: C, 73·5; H, 5·4; N, 4·7; $C_{18}H_{15}O_3N$ requires C, 73·7; H, 5·1; N, 4·8%).

(ii) *7-Amino-4-methyl-3-phenyl coumarin*

The acetate (4 g.) was heated with alcoholic hydrochloric acid (2:1, 100 ml.) for 2 hrs. and allowed to stand overnight. The compound that separated (3 g.) on recrystallisation from hot alcohol gave pale yellow needles, m.p. 280° C. (Found: C, 76·4; H, 5·5; N, 5·7; $C_{16}H_{13}O_2N$ requires C, 76·5; H, 5·2; N, 5·6%).

(iii) *7-Chloro-4-methyl-3-phenyl coumarin*

7-Amino-4-methyl-3-phenyl coumarin (0·5 g.) was dissolved in dil. sulphuric acid (30 ml., 5 N) by warming and diazotised with sodium nitrite (0·2 g. in 5 ml. water) at 0–5° C. After standing for 1 hr. the cold diazonium solution was added to an ice-cold solution of cuprous chloride (2 g. in 10 ml. conc. hydrochloric acid) with vigorous stirring. The reaction mixture was allowed to attain room temperature and then warmed

on the water-bath at 60° C. till the frothing subsided indicating the completion of the reaction. The 7-chloro-4-methyl-3-phenyl coumarin that separated was collected (0·4 g.) and on recrystallisation from alcohol gave colourless needles, m.p. 137° C. (Found: C, 71·0; H, 4·7; Cl, 13·1; $C_{16}H_{11}O_2Cl$ requires C, 71·0; H, 4·1; Cl, 13·1%).

(iv) *7-Bromo-4-methyl-3-phenyl coumarin*

The cold diazonium solution obtained from 7-amino-4-methyl-3-phenyl coumarin (0·5 g.) was added to a cold solution of cuprous bromide (2 g. in 10 ml. hydrobromic acid) gradually with mechanical stirring and the precipitate obtained (0·4 g.) was worked up as above. 7-Bromo-4-methyl-3-phenyl coumarin on recrystallisation from alcohol separated as lemon yellow needles, m.p. 129° C. (Found: C, 61·3; H, 3·7; Br, 24·9; $C_{16}H_{11}O_2Br$ requires C, 61·0; H, 3·5; Br, 25·4%).

(v) *7-Iodo-4-methyl-3-phenyl coumarin*

The cold diazonium solution obtained from 7-amino-4-methyl-3-phenyl coumarin (0·5 g.) was stirred with potassium iodide solution (2 g. in 10 ml. water) and the precipitate (0·5 g.) obtained was worked up as usual. The product on recrystallisation from alcohol gave greyish plates, m.p. 142° C. (Found: C, 53·6; H, 3·5; $C_{16}H_{11}O_2$ I requires C, 53·1; H, 3·1%).

Summary

7-Amino-4-methyl-3-phenyl coumarin has been prepared from 4-acetamido-*o*-hydroxy acetophenone. The corresponding halogen substituted coumarins, which are prepared through diazotisation and Sandmeyer reactions, have been found to be highly toxic to fish.

We are thankful to Mr. C. Venkata Ratnam for the microanalyses recorded in the paper.

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SYNTHETIC EXPERIMENTS IN THE BENZOPYRONE SERIES

Part LVII. Synthesis of 5 : 6 : 7 : 8 : 4′-Pentamethoxy Flavanone and Flavone: The Constitution of Ponkanetin

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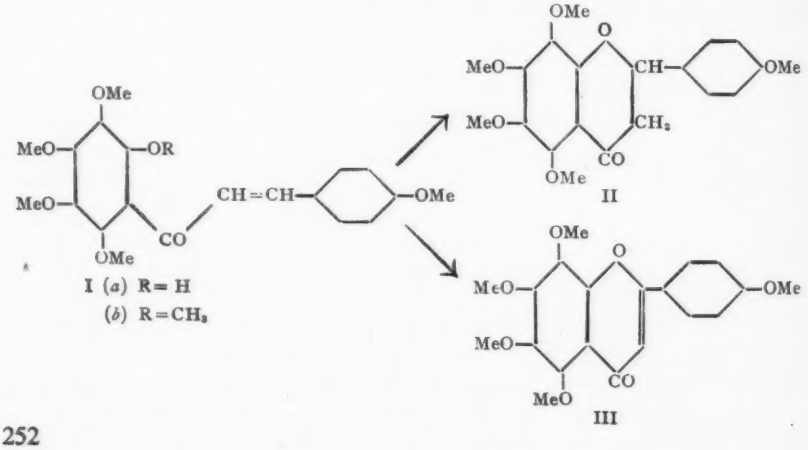
Ichikawa and Yamashita[1] reported the isolation of a new flavanone derivative melting at 152° from the peels of *Citrus poonensis* Hort, named it ponkanetin and gave it the constitution of 5 : 6 : 7 : 8 : 4′-pentamethoxy flavanone (II) as a result of detailed degradation study. The compound of this constitution has now been synthesised and is found to have a different melting point (108–09°). 5 : 6 : 7 : 8-Tetramethoxy flavanone which has been made earlier by Rao, Rao and Seshadri[2] was recorded to melt at 78–79°. The higher member is, therefore, more likely to have a melting point of 108° rather than 152°. Hence the structure assigned to ponkanetin may require reconsideration.

For the synthesis of the pentamethoxy flavanone (II), 2-hydroxy-3 : 4 : 5 : 6 : 4′-pentamethoxy chalkone (I *a*) is the intermediate. A good yield of it is obtained by the condensation of pentamethoxy benzene[3] with *p*-methoxy cinnamoyl chloride[4] in dry ether medium in the presence of aluminium chloride. The use of nitrobenzene reduces the yield considerably.



On treatment with alcoholic acid it smoothly undergoes isomeric change to 5:6:7:8:4′-pentamethoxy flavanone (II). It is also converted into the known 5:6:7:8:4′-pentamethoxy flavone (III)[5] by oxidation with selenium dioxide.

Experimental

2-Hydroxy-3:4:5:6:4′-pentamethoxy chalkone (*I a*).—Anhydrous aluminium chloride (10 g.) was dissolved in absolute ether (100 c.c.) with cooling in water and pentamethoxy benzene (5 g.) added. To the mixture which was cooled in water, a solution of *p*-methoxy cinnamoyl chloride (5·5 g.) in ether (50 c.c.) was slowly added during the course of 30 minutes with stirring. During this time, the dark coloured aluminium chloride complex of the chalkone began to separate. After standing overnight, the solvent was distilled off and to the residue was cautiously added water (200 c.c.) and concentrated hydrochloric acid (25 c.c.) and the mixture heated on a water-bath for five minutes. It was then cooled and extracted with ether (3×150 c.c.), the ether extract washed with dilute hydrochloric acid and shaken with excess of 10% aqueous sodium hydroxide. Acidification of the aqueous solution precipitated an oil which gradually solidified to an orange coloured mass on leaving it overnight in the refrigerator. It crystallised from petroleum ether as small orange yellow prisms and needles melting at 91–92°; 2·7 g. (Found: C, 63·8; H, 5·9; $C_{20}H_{22}O_7$ requires C, 64·2; H, 5·9%). It was easily soluble in common organic solvents and gave a brownish red colour with alcoholic ferric chloride. 2:3:4:5:6:4′-Hexamethoxy chalkone (I *b*), obtained by methylation with dimethyl sulphate and potassium carbonate in acetone medium, crystallised from petroleum ether as pale straw coloured long rhombohedral tablets; m.p. 105–06°; (Found: C, 65·4; H, 6·5; $C_{21}H_{24}O_7$ requires C, 64·9; H, 6·2%). The compound gave no colour with alcoholic ferric chloride.

5:6:7:8:4′-Pentamethoxy flavanone (*II*).—A solution of the above hydroxy chalkone (I *a*) (2 g.) in alcohol (100 c.c.) was treated with concentrated sulphuric acid (2·2 c.c.) and the resulting solution was refluxed for 30 hours in a water-bath. It was then diluted with water (100 c.c.) and distilled to remove most of the alcohol, the product taken up in a large excess of ether (400 c.c.) and the ether solution washed several times with cold aqueous 2% sodium hydroxide, dried and the ether distilled off. The residue left behind, crystallised from dry ether as colourless large flat needles and long rectangular plates melting at 108–09°; 1·2 g. (Found: C, 64·6; H, 5·4; $C_{20}H_{22}O_7$ requires C, 64·2; H, 5·9%). The compound developed a deep pink colour with magnesium and hydrochloric acid and with concentrated

nitric acid a deep orange red. It gave no colour with alcoholic ferric chloride.

5:6:7:8:4′-*Pentamethoxy flavone* (*III*).—The above chalkone (I *a*) (2 g.) was refluxed with selenium dioxide (1 g.) in amyl alcohol (25 c.c.) for 40 hours in an oil-bath at 140–45°. The insoluble mass was filtered off and the alcohol removed by steam distillation. The residue left behind solidified on leaving it in the refrigerator. It crystallised from ethyl acetate-petroleum ether mixture as long rectangular prisms and rods melting at 150–51°; 0·8 g. (Found: C, 63·9; H, 5·4; $C_{20}H_{20}O_7$ requires C, 64·5; H, 5·4%). Murti, Rao and Seshadri[5] who prepared it by a different route also reported the same melting point.

SUMMARY

5:6:7:8:4′-Pentamethoxy flavanone has been synthesised. The product is different from ponkanetin which was given the above structure earlier by Ichikawa and Yamashita.

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THERMAL EXPANSION OF POTASSIUM IODIDE

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(Communicated by Prof. R. S. Krishnan, F.A.SC.)

1. Introduction

A systematic investigation of the variation of thermal expansion coefficient of alkali halides with temperature at low temperatures has been undertaken by the author for the following reasons:—

Because of the simplicity of their structure the alkali halides occupy an important position in any theory of the solid state. Expansion measurements at low temperatures are not present for any of the alkali halides except sodium chloride.

The results of the measurements on sodium and potassium chlorides have already been reported (1955). The results for potassium bromide will be reported elsewhere. As the expansion of potassium iodide exhibits an anomalous behaviour at low temperatures it is reported here separately.

2. Previous Work

The thermal expansion of potassium iodide has been measured by Fizeau (1867–68) with his interference dilatometer He found a value of $42{\cdot}65\times10^{-6}$ for the linear expansion coefficient of potassium iodide at 40° C. The volume expansion eoefficient between 0 to 25° C. was determined by Baxter and Hawkins (1916) employing a pyknometer method. Henglein (1925) measured the volume expansion coefficient of potassium iodide between $-184/-79°$ and $-79/0°$ C. using a gas volumenometer to determine the density of the salt at these temperatures. Eucken and Dannohl (1934) measured the expansion of potassium iodide with the heterodyne beat method. But their values at room temperature are much lower than any of the values mentioned previously, the discrepancy amounting to as much as 20%.

Gott (1942) carried out a series of careful investigations on the macroscopic and lattice expansions of potassium iodide between 20 to 190° C. and found that the former was larger than the latter by about 14%. This discrepancy he attributed to the presence of Schottky defects in the lattice. But the number of such defects in the crystal deduced from other measurements are not sufficiently large to account for the observed discrepancy.

Connell and Martin (1951) have measured the lattice expansion of potassium iodide between 20 to 100° and 20 to 190° C. and found that their values of lattice expansion agreed well with the macroscopic values of Gott and were larger than the lattice expansion values of the same author.

3. METHOD OF MEASUREMENT AND OTHER DETAILS

The method of measurement both at high and at low temperatures has been indicated in previous papers of the author (1955 *a*, *b*).

Two different sets of spacers of potassium iodide were employed in this investigation. One set of three spacers was prepared from a specimen of potassium iodide grown from melt in our laboratory by Dr. Vedam and Mr. Gopalakrishnan to whom the author's thanks are due. The substance used for growing the crystal was an extra pure sample supplied by Merck and Co. The crystal was grown in a porcelain crucible and was annealed for two days. The crystal was clear and transparent.

The second set of three spacers was prepared from a specimen of pure potassium iodide supplied by Harshaw Chemical Co.

For measurements at high temperatures, the interferometric arrangement was heated up to 300° C. and cooled back to room temperature. The measurements were then made as the crystals were heated gradually.

Low temperature expansion was always measured after the high temperature experiment was made. This means the specimens were heated up to 300° C. many times before their low temperature expansion was measured.

On heating up to 300° C. the crystals did not exhibit any visible colouration.

For low temperature measurements, the specimens were first cooled to liquid-air temperature and the readings were taken as the specimens were heated gradually. The measurements were repeated for specimen 1, after the interferometric arrangement was taken apart and reset for a fresh run. Only one run was made for specimen 2. High temperature measurements were carried out for each set of specimens both before and after the low temperature measurements.

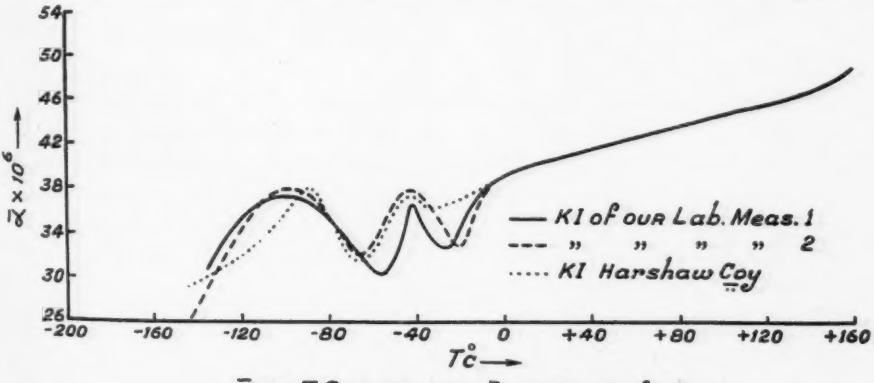
4. RESULTS

The results of these measurements are given in Table I. $\bar{\alpha}$ denotes the mean expansion coefficient over 25° C. intervals. The results are shown graphically in Fig. 1 for specimen 1 (measurements 1 and 2) and specimen 2.

TABLE I

Linear Expansion Coefficient ā of Potassium Iodide at Various Temperatures

Specimen 1 (Grown in our Laboratory) Heating 1		Heating 2		Specimen 2 (Harshaw) Heating 1	
T° C.	$\bar{a}\times10^6$	T° C.	$\bar{a}\times10^6$	T° C.	$\bar{a}\times10^6$
−135·1	30·6	−143·0	26·1	−142·1	29·1
−124·0	34·2	−130·7	30·6	−121·3	32·2
−113·2	36·3	−119·0	34·5	−106·7	34·4
−102·5	37·1	−109·0	36·7	− 96·4	36·5
− 93·3	37·1	− 98·0	37·5	− 88·3	37·2
− 84·4	36·7	− 88·1	37·5	− 80·3	36·2
− 73·2	33·9	− 78·3	35·2	− 69·2	31·9
− 63·5	31·2	− 68·1	32·3	− 57·1	32·9
− 52·0	30·4	− 58·0	33·9	− 46·8	36·7
− 42·2	36·5	− 48·4	37·1	− 32·1	36·5
− 32·3	33·6	− 37·9	37·1	− 21·6	37·7
− 22·5	32·9	− 27·9	34·9	− 12·2	38·7
− 13·0	36·7	− 18·2	33·9	2·1	40·0
− 4·1	38·4	4·0	39·6		
23·0	40·5				
46·4	41·9			46·8	42·0
63·3	43·1			69·2	43·1
88·9	43·5			90·9	44·1
105·4	44·7			112·3	44·8
121·4	45·1			132·9	46·5
133·7	46·9			153·9	47·9
147·7	47·1				



ᾱ VS. T CURVES FOR POTASSIUM IODIDE

FIG. 1

5. DISCUSSION

An examination of the results given in Table I and the graphs in Fig. 1 reveals the following important features:

1. Below room temperature, the thermal expansion of potassium iodide behaves in an anomalous way. The expansion coefficient *versus* temperature curve for this crystal exhibits two maxima and two minima. The maxima occur at average temperatures of $-95°$ and $-40°$ C. The positions of the minima occur at $-60°$ and $-25°$ C. The maxima and minima show slight shifts along the temperature axis from one curve to another. The values of these expansion coefficients at these maxima and minima are nearly the same for all the curves. The expansion coefficient ranges from $37{\cdot}5\times10^{-6}$ at the maxima to about $30{\cdot}8\times10^{-6}$ at the minima—a difference of about 20%. The experimental error of these measurements is less than 3%.

All the measurements were carried out as the specimens were heated from liquid-air temperatures to room temperatures. Measurements were attempted on the Harshaw specimen (specimen 2) as it was cooled from room temperature to liquid-air temperature. But because of the initial rapid cooling reliable values were not obtained till about $-50°$ C. The values of the expansion coefficient while cooling also exhibited a very sharp maximum at $-100°$ C. coinciding with the maximum at about the same temperature obtained while heating the specimens.

Above 0° C. all the curves merge with the high temperature expansion curve.

The values of α observed by Henglein (1925) between $-185/-79°$ and $-79/0°$ C. were mean values and in view of the present observations do not have any significance.

The specific heat of potassium iodide has been determined by the method of mixtures by Korf (1911) between $-184/-79°$ and $-79/0°$ C. By the nature of the experiment, only mean values of the specific heat over these temperatures could have been obtained by him and so any small anomaly in the specific heat could not be detected. Bronstead (1914) has measured the specific heat of potassium iodide at 10° C. This is a temperature region which is not of any interest to us.

The specific heat of potassium iodide has been measured from 10 to 273° K. by Clusius, Goldmann and Perlick (1949). Unfortunately that paper is not available to us. But from the summary of the paper given in *Chemical Abstracts* (1950) it appears that no anomaly has been reported

between these temperatures. For want of a precise knowledge of their values, we cannot make any comparison between thermal expansion and specific heat values.

2. Above room temperature the macroscopic values of expansion of the two specimens are identical within the limits of experimental error (3%). The high temperature values are completely reproducible and are not affected by cooling the specimens to liquid-air temperatures and heating them back again to room temperature. Also the present experiments on potassium iodide above room temperature and all the other measurements except that of Eucken and Dannohl agree in obtaining a value of over 40×10^{-6} for $\bar{\alpha}$. Eucken and Dannohl's (1934) values are too low and are definitely affected by systematic errors.

Finally from the expansion *versus* temperature curve we see that the length of the spacers do not show any abrupt changes but vary continuously with temperature from -140 to $0°$ C. This behaviour appears to point to a homomorphous transition (Zwikker, 1954) in this crystal. Bergmann (1942), in his experiments on the variation of the solubilities of potassium chloride, bromide and iodide with temperature, found kinks in the curves at 27, 22 and 11° C. respectively. He postulated a homomorphous transition from α to β type for these salts at these temperatures. But he found no X-ray evidence for any change in the lattice structure at these temperatures. Neither has the present author found any abnormality in the expansion-temperature curves of potassium chloride and bromide at these temperatures. In potassium iodide also the anomalous behaviour in expansion is observed only below 0° C.

Sharma (1952) has observed the change in intensity with temperature of thermoluminiscence bands of a crystal of potassium iodide coloured by cathode-ray bombardment at liquid oxygen temperature. He observed four peaks in the curve at 110 to 120° K., 158° K., 176° K. and 235° K. The last two peaks occur at temperatures almost coincident with the two maxima in the expansion observed by the author at $-95°$ and $-40°$ C. This coincidence appears to be fortuitous.

Further investigations on X-ray diffraction, Raman effect, etc., in potassium iodide at low temperatures, are called for before one could postulate any hypothesis as to the nature of the anomaly.

In conclusion, the author wishes to express his gratitude to Prof. R. S. Krishnan for his interest and encouragement during the course of this investigation and for having provided the specimen of potassium iodide grown by Harshaw Chemical Co.

SUMMARY

The thermal expansion of two different specimens of potassium iodide was measured from liquid-air temperatures to 150° C. One specimen was melt grown in our laboratory and other was supplied by Harshaw Chemical Co.

The expansion coefficient for the two specimens above room temperature are identical with one another. The high temperature expansion was not influenced by heat or cold treatment of the specimens.

Below 0° C. the thermal expansion of potassium iodide exhibits an anomalous behaviour. Two maxima at − 95° and − 40° C. and two minima at − 60° and − 25° C. have been observed. As the length of the specimen varies continuously with the temperature, the transition in this crystal appears to be homomorphous.

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STUDIES IN THE ISOQUINOLINE SERIES

Part II. Oxidation of 1-Benzyl-3 : 4-Dihydroisoquinolines

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The remarkable ease of oxidation of 1-benzyl-3 : 4-dihydroisoquinolines was first observed by Buck, Haworth and Perkin[1] who found that exposure to air of alcoholic solutions of 3 : 4-dihydro-6 : 7-dimethoxy-1-(3 : 4-dimethoxybenzyl)- and 3 : 4-dihydro-6 : 7-dimethoxy-1-(3 : 4-methylenedioxybenzyl)-isoquinolines caused their conversion to the corresponding 1-benzoyl-3 : 4-dihydroisoquinolines, the latter also being obtained by oxidation of the 1-benzyl-3 : 4-dihydro bases with iodine. Similar auto-oxidations of 1-benzyl-3 : 4-dihydroisoquinolines have been reported by other authors, the auto-oxidations proceeding with such facility that the 1-benzoyl-3 : 4-dihydroisoquinolines are the only products obtained in the Bischler-Napieralsky reaction.[2, 3]

Gulland and Haworth[4] observed however that 3 : 4-dihydro-1-(2-nitrobenzyl)-isoquinolines are remarkably stable to auto-oxidation. We have also found that 3 : 4-dihydro-6 : 7-dimethoxy-1-(3-methoxy-2-nitrobenzyl)-isoquinoline was recovered unchanged after prolonged exposure of the alcoholic solution to air or after treatment with iodine. Apparently, substituents in the benzyl portion of the molecule control the ease of oxidation and in this paper we present a systematic study of the oxidation of various 1-benzyl-3 : 4-dihydroisoquinolines, which were synthesised for this purpose and whose properties were recorded in an earlier paper.[5]

Exposure to air of alcoholic solutions of these bases resulted in auto-oxidation to the corresponding 1-benzoyl-3 : 4-dihydroisoquinolines (Table I) with one exception. The products gave a green colour with hot acetic anhydride, a test specific for 1-benzoyl-3 : 4-dihydroisoquinolines.[1] That these were not 1-benzoylisoquinolines was shown by the oxidation of 3 : 4-dihydro-6 : 7-dimethoxy-1-(4-methoxybenzyl)-isoquinoline with alcoholic potassium hydroxide to 6 : 7-dimethoxy-1-(4-methoxybenzoyl)-isoquinoline which was shown to be different from the product of auto-oxidation. Only 3 : 4-dihydro-6 : 7-dimethoxy-1-(2-nitrobenzyl)-isoquinoline was recovered unchanged after prolonged exposure of its alcoholic solution to air. The

TABLE I

1-Benzoyl-3 : 4-Dihydro-6 : 7-Dimethoxyisoquinolines

Substituent in benzoyl residue	Method of preparation*	BASE						PICRATE					
		Mol. formula	M.P. °C.	Found %		Required %		Mol. formula	M.P. ° C.	Found %		Required %	
				C	H	C	H			C	H	C	H
4-OMe	*a*, *b*	$C_{19}H_{19}O_4N$	110–12	69·83	6·02	70·15	5·85	$C_{25}H_{22}O_{11}N_4$	220 (decomp.)	54·41	4·02	54·16	3·97
2-OMe	*a*, *b*							$C_{25}H_{22}O_{11}N_4$	180	54·03	4·05	54·16	3·97
4-Me	*a*, *b*	$C_{19}H_{19}O_3N$	93–95	73·41	6·13	73·77	6·15	$C_{25}H_{22}O_{10}N_4$	183 (sinters at 172)	55·54	4·26	55·76	4·09
2-Me	*a*, *b*							$C_{25}H_{22}O_{10}N_4$	172 (decomp.)	55·68	4·07	55·76	4·09
2-Br	*b*	$C_{18}H_{16}O_3NBr$	159–60 (decomp.)	57·81	4·10	57·76	4·28	$C_{24}H_{19}O_{10}N_4Br$	159–60 (decomp.)	47·89	3·01	47·76	3·15
3-Br	*b*	$C_{18}H_{16}O_3NBr$	126–27	57·88	4·69	57·76	4·28	$C_{24}H_{19}O_{10}N_4Br$	173–74 (decomp.)	47·48	3·54	47·76	3·15
4-Br	*b*	$C_{18}H_{16}O_3NBr$	132	57·31	4·45	57·76	4·28	$C_{24}H_{19}O_{10}N_4Br$	193–94 (shrinks above 180)	48·09	3·21	47·76	3·15
3-Nitro	*b*	$C_{18}H_{14}O_5N_2$	175–76	63·82	4·87	63·53	4·71	$C_{24}H_{19}O_{12}N_5$	155–57 (sinters at 147	50·24	3·69	50·62	3·34

* *a* iodine oxidation.
b air oxidation.

corresponding 4-nitrobenzyl compound under the same conditions yielded a resin from which no pure product could be isolated.

Air oxidations must necessarily proceed by a free radical mechanism. It is not surprising therefore that these auto-oxidations proceed in all cases irrespective of the electrical nature of the substituents in the benzyl portion of the molecule, though it is not clear why 3 : 4-dihydro-6 : 7-dimethoxy-1-(2-nitrobenzyl)-isoquinoline alone should resist air oxidation.

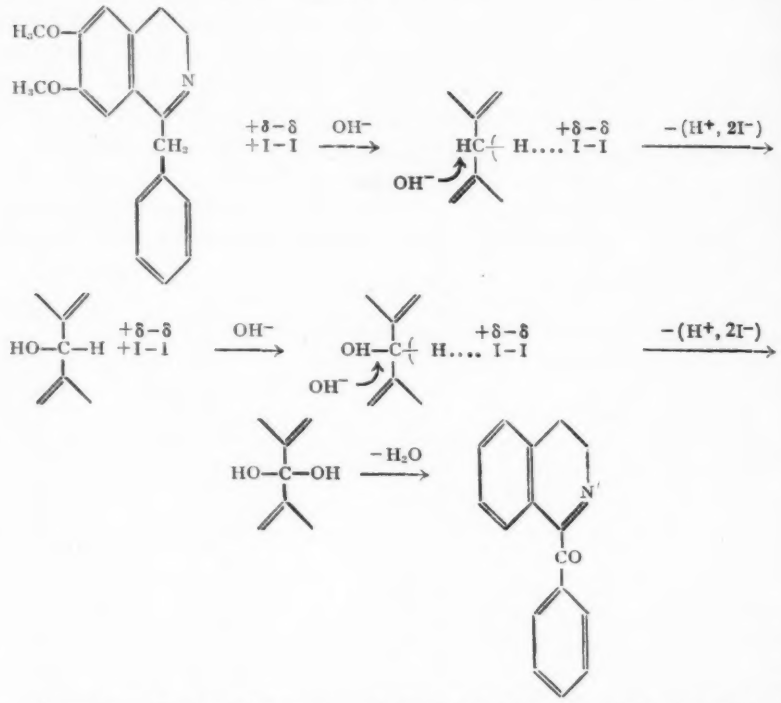
It is of interest that oxidation with iodine yields the same product as in the auto-oxidation, although they must proceed by different mechanisms. In order to obtain a semi-quantitative idea of the relative ease of oxidation of the various 1-benzyl-3 : 4-dihydroisoquinolines, equimolecular quantities were treated with a known excess of a standard solution of iodine for the same period and the excess iodine then determined. The results of the study are presented in Table II. It is seen that 3 : 4-dihydro-6 : 7-dimethoxyisoquinoline is unaffected by iodine, showing that iodine does not bring

TABLE II

Reaction between Alcoholic Iodine and 3 : 4-*Dihydroisoquinolines*

6 : 7-Dimethoxy-3 : 4-dihydroisoquinoline with substituent at 1-position	Wt. of hydrochloride in gm.	Thiosulphate (0·07658 N) equivalent of iodine uptake c.c.	Iodine uptake %
Nil	0·1572	0	0
Benzyl	0·1989	4·60	14·1
2-Bromobenzyl	0·2479	0	0
3-Bromobenzyl	0·2449	4·75	14·7
4-Bromobenzyl	0·2476	4·70	14·1
2-Methylbenzyl	0·2098	6·60	20·0
3-Methylbenzyl	0·2282	5·80	16·0
4-Methylbenzyl	0·2051	14·35	44·0
2-Methoxybenzyl	0·2158	16·50	50·9
3-Methoxybenzyl	0·2127	6·55	20·5
4-Methoxybenzyl	0·2158	23·50	72·5
2-Nitrobenzyl	0·2395	1·70	4·9
3-Nitrobenzyl	0·2380	4·50	13·7
4-Nitrobenzyl	0·2334	3·85	11·5
4-Cyanobenzyl	0·2146	3·50	10·7

about dehydrogenation. The iodine taken up by various 1-benzyl-3:4-dihydroisoquinolines must be solely utilised for oxidation of the methylene group. In four cases where there was considerable uptake of iodine, the 1-benzoyl-3:4-dihydro bases were isolated in pure condition. In three cases where iodine uptake was negligible, the original compounds were recovered. In two cases where the iodine uptake was about 20%, only impure compounds could be isolated.



A study of the results presented in Table II reveals that the oxidation of 1-benzyl-3:4-dihydroisoquinolines by iodine is influenced both by steric and polar effects. Thus the uptake of iodine by 3:4-dihydro-1-(2-methylbenzyl)-isoquinoline is considerably less than that of 3:4-dihydro-1-(4-methylbenzyl)-isoquinoline. The presence of a nitro group in any position of the benzyl group inhibits oxidation by iodine, the effect being maximum when *ortho*- to the methylene group. A bromine atom *ortho*- to the methylene group completely inhibits oxidation, whereas when present in other positions, the iodine consumption is of the same order as for the unsubstituted compound. A plausible mechanism for the oxidation of

1-benzyl-3 : 4-dihydroisoquinolines may be postulated as below involving the direct approach of a polarised iodine molecule or a I^+ ion to the methylene group.

It is unlikely that a hypoiodite ion is involved since in blank experiments with alcoholic iodine and potassium acetate, very little iodine was consumed.

Experimental Procedure

Determination of the relative ease of oxidation of 3 : 4-*dihydro*-6 : 7-*dimethoxyisoquinolines with iodine*

The base hydrochloride (*ca.* 0·0006 mole) was weighed accurately and dissolved in alcohol (10 c.c.). An aqueous solution of potassium acetate (3 c.c., 1·82 N) and an alcoholic solution of iodine (20 c.c., 0·22 N) were pipetted into the solution of the hydrochloride and the mixture was kept for 1 hr. with occasional shaking. Unreacted iodine was then estimated against standard sodium thiosulphate solution with starch indicator. The difference between this and a blank gave the thiosulphate equivalent of the iodine consumed by the dihydroisoquinoline. The ratio of iodine uptake to the quantity of iodine required for complete oxidation of the benzyldihydroisoquinoline to benzoyldihydroisoquinoline, *i.e.*, 3 moles of iodine per 1 mole of the compound, was computed. The results are presented in Table II.

1-*Benzoyl*-3 : 4-*dihydro*-6 : 7-*dimethoxyisoquinolines*

(*a*) *By oxidation of the benzyl bases with iodine.*—A solution of the base hydrochloride (0·2 g.) in alcohol (5 c.c.) containing sodium acetate (1 g.) was treated with iodine (0·3 g.) in alcohol (10 c.c.). After 1 hr. excess iodine was discharged by addition of the requisite quantity of sodium thiosulphate. Addition of a solution of picric acid (0·5 g.) in water precipitated the picrate, which was collected and recrystallised from alcohol.

1-(4-Methoxybenzoyl)-, 1-(2-methoxybenzoyl)-, 1-(4-methylbenzoyl)-, and 1-(2-methylbenzoyl)-3 : 4-dihydro-6 : 7-dimethoxyisoquinolines were obtained by this precedure in yields of 78, 63, 59 and 43% respectively. Almost quantitative recovery of the starting material was obtained in the cases of 3 : 4-dihydro-6 : 7-dimethoxyisoquinoline, and 1-(2-bromobenzyl)- and 1-(2-nitrobenzyl)-3 : 4-dihydro-6 : 7-dimethoxyisoquinolines. Impure products were obtained in the cases of 1-(3-bromobenzyl)- and 1-(4-bromobenzyl)-3 : 4-dihydro-6 : 7-dimethoxyisoquinolines. These are probably mixtures of the original and the oxidised bases.

(*b*) *By air oxidation.*—A solution of the benzyldihydro base (1 g.) in alcohol was kept exposed to air for several days. The sticky solid so obtained gave the benzoyldihydro derivative on repeated crystallisation from alcohol. 3 : 4-Dihydro-6 : 7-dimethoxy-1-(2-nitrobenzyl)-isoquinoline was recovered completely unchanged by this method, while the corresponding (4-nitrobenzyl)—base gave a resin which could not be crystallised or made to yield suitable derivatives.

The benzoyldihydro bases are listed in Table I.

6 : 7-*Dimethoxy*-1-(4-*methoxybenzoyl*)-*isoquinoline*

A solution of 3 : 4-dihydro-6 : 7-dimethoxy-1-(4-methoxybenzyl)-isoquinoline (0·7 g.) was digested on a water-bath with methanolic potassium hydroxide (10%, 10 c.c.) for 4 hr. with addition of methanol at intervals. The sticky mass was washed with water and crystallised from alcohol to yield the benzoyl base as colourless needles (0·2 g.), m.p. 145–46° (Found: C, 70·2; H, 5·5; $C_{19}H_{17}O_4N$ requires C, 70·6; H, 5·2%).

SUMMARY

The oxidation of several 1-benyzl-3 : 4-dihydroisoquinolines in alcoholic solution by air and by iodine has been studied. A mechanism is proposed for the action of iodine on these bases.

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POLAROGRAPHIC BEHAVIOUR OF THIONATES

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Furness[1] has shown that trithionate can be reduced polarographically and that the reduction waves can be used for the estimation of trithionate. Later Furness and Davies[2] extended this work to include tetra- and pentathionates. They have suggested tentative procedures for the determination of tetrathionate in presence of dithionate, trithionate, sulphite or thiosulphate but could not recommend any procedure for estimating tetrathionate in presence of pentathionate. Teisuke Murayama[3] has studied di-, tri- and tetrathionates in one-tenth normal potassium chloride and one-tenth normal barium chloride. In the present paper a detailed account is given of the polarographic estimation of thionates.

Experimental

The polarograms were recorded automatically with the Cambridge Instrument Company Polarograph. In all the measurements, the potentials were measured with a saturated calomel electrode as the half-cell. The polarographic cell was maintained at 30° C. $\pm$ 0·1° C. in a well grounded water thermostat. Oxygen was removed from the solutions with a stream of purified hydrogen. The '*m*' values of the dropping mercury electrodes used were 1·370, 1·227 and 1·150 mg. per second and the drop time was adjusted to 5 seconds in normal potassium chloride. The potentiometric drum was run at a low speed to obtain negligible falsification of the half-wave potential. Heights of the polarographic waves, and the values of $E_{\frac{1}{4}}$ and $(E_{\frac{3}{4}} - E_{\frac{1}{4}})$ were measured by the method developed in this laboratory (which is an improvement of the method suggested by Taylor[4]) and the values are given after incorporating residual current and *i*R drop corrections respectively.

Di-, tri-, tetra-, and pentathionates were prepared by standard methods.[5-7] The concentration of the solutions prepared was checked up by the methods developed by Kurtenacker and collaborators and Glasstone and Hickling.[8]

Results

1. *Dithionate*

Barium dithionate was used for polarographic analysis. It was noticed that it could not be reduced below the discharge potential of sodium. Furness

and Davies[2] have stated that dithionate does not give a reduction wave in ammonium dihydrogen phosphate solutions.

2. *Trithionate*

As the half-wave potential of trithionate is of the order of $-1{\cdot}2$ V, its polarographic behaviour can be studied only above pH 7. But high pH values (above 11), however, produce decomposition. Furness[1] has stated that trithionate gives good polarographic waves in 0·5 M diammonium hydrogen phosphate and 1 M ammonia. But Furness and Davies[2] while applying the method to estimate a mixture of tri- and tetrathionates have merely used diammonium hydrogen phosphate evidently due to the influence of ammonia on the tetrathionate wave.

Preliminary work in this laboratory has shown that better polarograms are obtained by using potassium chloride or iodide than by using diammonium hydrogen phosphate. It has also been noticed that inclusion of 50% alcohol in base solutions containing potassium chloride or iodide has a profound influence on the characteristics of the waves. In the following investigation a detailed account in unbuffered solutions containing potassium chloride, potassium iodide (with and without alcohol), and ammonium chloride (1 M)-ammonium hydroxide (1 M) are presented.

Some of the polarograms obtained with trithionate in potassium chloride are presented in Figs. 1 and 2. Curves with potassium iodide are similar in nature. From the figure (also Table I) it can be seen that the half-wave potential becomes more positive with an increase in the concentration of the salt and the diffusion current plateau becomes well defined. It is also clear that the inclusion of 50% alcohol in the base solutions lessens the long drawn-out nature of the polarographic waves. The results of the analysis of the waves are presented in Table I, from which, it is clear that (i) the half-wave potential decreases with an increase in salt concentration; (ii) the $E_{\frac{3}{4}} - E_{\frac{1}{4}}$ value is of the order of 0·19 V, showing that the reduction process is irreversible in character.

The effect of concentration of trithionate on the diffusion current constant under a variety of conditions is included in Table II. It has been noticed that in aqueous and alcoholic potassium iodide and alcoholic potassium chloride the C — V curves are characterized by a small maximum which appears at higher concentrations of trithionate. The maximum, however, is suppressed by adding 0·01% gelatin but in aqueous potassium iodide solutions the diffusion current plateau is not perfectly flat and, hence, not quite suited for analytical purposes.

TABLE I. *Polarographic behaviour of trithionate in presence of different base solutions*

Base solution used	Concn. of the base solution (Molar)	Half-wave potential $E_{\frac{1}{2}}$ *vs.* S.C.E. (Volt)	$E_{\frac{3}{4}}-E_{\frac{1}{4}}$ (Volt)	Remarks
Diammonium hydrogen phosphate ..	0·05	−1·638	0·171	Diffusion current plateau is not good.
	0·50	−1·354	0·194	do.
	1·00	−1·274	0·197	do.
	2·00	−1·144	0·194	do.
Disodium hydrogen phosphate ..	0·80	−1·422	0·212	do.
Potassium chloride ..	0·10	−1·652	0·184	
	1·00	−1·390	0·202	
	2·00	−1·243	0·198	
Potassium iodide ..	0·05	−1·724	0·189	Split-waves very close to each other.
	0·50	−1·340 −1·563	..	
	1·00	−1·177 −1·392	..	
	2·00	−1·232	0·154	One wave.
Potassium chloride in 50% alcohol ..	1·00	−1·346	0·067	Well marked diffusion current plateau.
Potassium iodide in 50% alcohol (0·01% gelatin) ..	1·00	−1·346	0·098	do.

TABLE II. *Effect of concentration of trithionate on the diffusion current constant*

Base solution used	Concn. of trithionate (Millimolar)	$m^{\frac{2}{3}}t^{\frac{1}{6}}$	Diffusion current constant $\frac{i_d}{Cm.^{\frac{2}{3}}t^{\frac{1}{6}}}$
1 M potassium iodide in 50% alcohol	1·560	1·393 mg.$^{\frac{2}{3}}$ sec.$^{-\frac{1}{2}}$ at −1·57 V *vs.* S.C.E.	2·90
	7·800	do.	2·93
1 M potassium chloride in 50% alcohol	1·560	do.	2·80
	7·800	do.	2·76
2 M aqueous potassium chloride solution ..	1·135	1·449 mg.$^{\frac{2}{3}}$ sec.$^{-\frac{1}{2}}$ at −1·70 V *vs.* S.C.E.	4·07
	2·270	do.	4·05
	5·675	do.	4·03

Ammonium chloride (1 *M*)-*ammonium hydroxide* (1 *M*).—In this base solution 0·01% gelatin is added to eliminate the maxima. The half-wave potential is $-1{\cdot}387$ V and $E_{\frac{3}{4}} - E_{\frac{1}{4}}$ is 0·184 V showing the irreversibility of the electrode process. The diffusion current constant is 4·2. The diffusion current plateau, however, is not so well marked as in the case of potassium chloride or iodide.

3. *Tetrathionate*

The polarography of tetrathionate can conveniently be studied as the wave appears below the final current rise due to hydrogen discharge. Furness and Davies[2] have used ammonium dihydrogen phosphate with quinoline as maximum suppressor. They have also suggested tentative procedure for the analysis of tetrathionate and trithionate by using diammonium hydrogen phosphate (1 M) with gelatin (0·01%). However, it has been noticed in this laboratory that tetrathionate in 1 M diammonium hydrogen phosphate gives a horizontal stretch of the diffusion current plateau from $-0{\cdot}35$ V to $-0{\cdot}90$ V *vs.* S.C.E. But thereafter, there is a progressive increase in the diffusion current till the final rise of current is reached. Such curves cannot therefore be employed for accurate estimation of the trithionate in presence of tetrathionate. An alternate procedure has been developed in this laboratory for a more accurate polarographic estimation of tetrathionate using different reagents.

(1) *Hydrochloric acid.*—It has been noticed that in 1 N hydrochloric acid, maximum does not appear. In 0·2 M hydrochloric acid a rounded maximum appears on the C — V curves which can be suppressed by 0·02% gelatin. Some of the polarograms are presented in Fig. 3. The results of analysis are given in Table III from which it is clear that the reduction proceeds irreversibly.

Table IV gives the relationship between the diffusion current constant and the concentration of tetrathionate, and indicates that this medium can be used for analytical purposes.

(2) *Buffered solutions.*—Some of the polarograms obtained in buffer solutions have been shown in Fig. 4. 0·02% gelatin has been incorporated in the base solutions. It is seen that the maxima cannot be eliminated. The half-wave potentials have no significance when the maximum is present.

(3) *Potassium chloride and iodide.*—Polarograms of tetrathionate are marked with a characteristic humpy maxima at $-0{\cdot}6$ V in neutral potassium chloride solutions (0·1 M). Addition of gelatin (0·01%), however, lessens the maxima but does not completely remove it. Increase of potassium

TABLE III. *Polarographic behaviour of tetrathionate in presence of different base solutions*

Base solution	Concn. of the base solution (Molar)	Remarks
1. Potassium iodide ..	0·016	Sensitivity: *s*/70. Total height = 29 divisions. Small hump at − 0·6 V *vs.* S.C.E. Main waves are two and they are very near each other at about − 1·3 to − 1·4 V. Highly extended waves.
2. Potassium iodide ..	0·04	Sensitivity: *s*/70. Total height = 29 divisions. Hump decreases in size giving an imperfect wave at about − 0·56 V *vs.* S.C.E. Second wave is drawn out as in the previous case. Two waves are very close to each other about − 1·3 to − 1·4 V.
3. Potassium iodide ..	0·06	Sensitivity: *s*/70. Height of the first wave = 4 divisions. Height of the second wave = 25 divisions. First wave very clearly marked. $E_{\frac{1}{2}}$ about − 0·60 V *vs.* S.C.E. Second wave is drawn out. $E_{\frac{1}{2}}$ = − 1·32 V *vs.* S.C.E.
4. Potassium iodide ..	0·08	Sensitivity: *s*/70. Height of the first wave = 8·5 divisions and that of the second wave 20·5 divisions. Two clear waves. $(E_{\frac{1}{2}})_1$ = − 0·65 V *vs.* S.C.E. and $(E_{\frac{1}{2}})_2$ = − 1·17 V *vs.* S.C.E.
5. Potassium iodide ..	0·16	Sensitivity: *s*/70. Height of the first wave 12·3 divisions and that of the second wave 17·5 divisions. Diffusion current of the first wave less clearly marked. $(E_{\frac{1}{2}})_1$ = − 0·73 V *vs.* S.C.E. and $(E_{\frac{1}{2}})_2$ = − 1·17 V *vs.* S.C.E.
6. Potassium iodide ..	0·30	Sensitivity: *s*/70. Total height 29 divisions. Second wave is apparent only in the form of an elongated bend before the diffusion current plateau. $(E_{\frac{1}{2}})_1$ = − 0·70 V *vs.* S.C.E.
7. Potassium iodide ..	0·50 1·00 3·00	Sensitivity: *s*/70. Total height 29 divisions. The bend before the diffusion current plateau becomes sharper as the concentration of potassium iodide increases and the half-wave potentials are about − 0·75 V, − 0·78 V. and − 0·80 V. *vs.* S.C.E. in 0·50 M, 1·00 M and 3·00 M solutions respectively.

TABLE III—(*Continued*)

Base solution	Concn. of the base solution (Molar)	Remarks
8. Hydrochloric acid ..	1·00	$E_{\frac{1}{2}} = -0{\cdot}285$ V *vs.* S.C.E. $E_{\frac{3}{4}} - E_{\frac{1}{4}} = 0{\cdot}143$ V.
9. Hydrochloric acid + 0·02% gelatin ..	0·20	$E_{\frac{1}{2}} = -0{\cdot}254$ V *vs.* S.C.E. $E_{\frac{3}{4}} - E_{\frac{1}{4}} = 0{\cdot}125$ V.
10. Potassium chloride + 0·01% gelatin	0·10 0·50 1·00 3·00	The main wave is at about $-0{\cdot}3$ V *vs.* S.C.E. The value of the half-wave potential decreases with increasing concentration of potassium chloride. After a certain voltage, the diffusion current increases slowly reaching a horizontal plateau before the final current rise.

chloride concentration to 3·0 M gives a horizontal stretch of the diffusion current plateau ($E_{\frac{1}{4}} = -0{\cdot}32$ V *vs.* S.C.E.) but at higher concentrations of the tetrathionate, the maximum again makes its appearance. At intermediate concentrations of potassium chloride there is a horizontal stretch from $-0{\cdot}6$ V to $-1{\cdot}0$ V and thereafter the diffusion current plateau is by no means horizontal but goes on rising and attains a constant value at about 0·2 to 0·3 V more positive than the final current rise. From these considerations it is clear that aqueous potassium chloride solutions cannot be employed for the estimation of tetrathionate.

It is surprising that in aqueous alcoholic solutions of potassium chloride or iodide (even without the addition of gelatin) the maximum (at about $-0{\cdot}6$ V *vs.* S.C.E.) should have completely disappeared giving place to a single wave at about $-1{\cdot}2$ V *vs.* S.C.E. Table IV gives the relationship between diffusion current constant and the concentration of tetrathionate. The constancy of the values of the diffusion current constant indicates that the method can be used for the estimation of tetrathionate.

Figures 5 and 6 give some of the polarograms showing the effect of potassium iodide on the polarographic behaviour of tetrathionate. The corresponding data are recorded in Table III. For analytical purposes, 1 M potassium iodide can be used as the base electrolyte, as indicated by the constancy of the diffusion current constants (Table IV).

As in the case of potassium chloride, potassium iodide in 50% alcohol gives a polarographic wave at about $-1{\cdot}2$ V *vs.* S.C.E.; 0·01% gelatin has

TABLE IV

Effect of concentration of tetrathionate on the diffusion current constant

Base solution used	Concn. of tetrathionate (Millimolar)	$m^{\frac{2}{3}} t^{\frac{1}{6}}$	Diffusion current constant $\frac{i_d}{Cm^{\frac{2}{3}} t^{\frac{1}{6}}}$
1 M aqueous potassium iodide solution ..	0·586	1·602 mg.$^{\frac{2}{3}}$ sec.$^{-\frac{1}{2}}$ at −1·27 V *vs.* S.C.E.	4·14
	2·344	do.	4·08
	4·102	do.	4·06
	5·274	do.	4·11
1 M hydrochloric acid ..	0·304	1·643 mg.$^{\frac{2}{3}}$ sec.$^{-\frac{1}{2}}$ at − 0·57 V *vs.* S.C.E.	3·90
	0·608	do.	3·91
	1·520	do.	3·78
	3·040	do.	3·79
0·2 M hydrochloric acid*	0·320	1·624 mg.$^{\frac{2}{3}}$ sec.$^{-\frac{1}{2}}$ at −0·70 V *vs.* S.C.E.	3·82
	0·801	do.	3·82
	1·602	do.	3·75
	3·200	do.	3·91
1 M potassium chloride in 50% alcohol ..	0·479	1·393 mg.$^{\frac{2}{3}}$ sec.$^{-\frac{1}{2}}$ at −1·57 V *vs.* S.C.E.	2·93
	2·393	do.	2·90
	4·785	do.	2·90
	9·570	do.	2·85
1 M potassium iodide in 50% alcohol† ..	0·479	do.	2·90
	9·570	do.	2·86

* 0·02% gelatin has been added to the base solution.
† 0·01% gelatin has been added to the base solution.

to be used to prevent the appearance of maxima at higher concentrations of tetrathionate. The results given in Table IV show that this medium can be employed for the estimation of tetrathionate.

(4) *Ammonium chloride—Ammonium hydroxide.*—When the polarograms of tetrathionate are taken in this media the maximum at − 0·6 V completely disappears and a wave appears at − 1·45 V *vs.* S.C.E. The wave-height however is not proportional to concentration. The half-wave potential of the trithionate wave under similar conditions is found to be − 1·38 V *vs.* S.C.E. It can therefore be concluded that this wave is due to the reduction of tetrathionate itself and not due to the instantaneous conversion of tetrathionate to trithionate.

4. *Pentathionate*

The base solutions in which the pentathionate can be studied are limited. It is well known that in neutral, weakly acid or basic solutions pentathionate gives a precipitate of sulphur. Hence acidic solutions are best suited for its determination. In 1 N hydrochloric acid the pentathionate gives a reduction wave, the half-wave potential of which is $-0{\cdot}211$ V *vs.* S.C.E. As the concentration of hydrochloric acid is decreased it shifts slightly to more negative potentials ($-0{\cdot}226$ V *vs.* S.C.E. in 0·2 M HCl). No maximum appears in these solutions. The slope of the wave is high, the value of $E_{\frac{3}{4}} - E_{\frac{1}{4}}$ being equal to 0·013 V. Furness and Davies[2] have noticed a polarographic wave in 1 M phosphoric acid solutions and state that it is not possible to estimate the tetrathionate when it is present in presence of pentathionate, due to the nearness of their half-wave potentials. However, it has been noticed in the present investigation that the half-wave potential of tetrathionate in hydrochloric acid is shifted beyond the final current rise while with pentathionate the reduction wave appears even in presence of 50% alcohol in the base solution. When the concentration of hydrochloric acid is 0·2 N, the C—V curves are characterised by a rounded wave. In higher concentrations of hydrochloric acid, the hump disappears. Consequently, 2 N hydrochloric acid has been incorporated in 50% alcoholic solutions. Table V shows the effect of concentration of pentathionate on the diffusion current constant under a variety of conditions. The results indicate that the various media can be employed for the polarographic estimation of pentathionate.

TABLE V

Effect of concentration of pentathionate on the diffusion current constant

Base solution used	Concn. of pentathionate (Millimolar)	$m^{\frac{2}{3}}\,t^{\frac{1}{6}}$	Diffusion current constant $\frac{id}{Cm^{\frac{2}{3}}\,t^{\frac{1}{6}}}$
0·2 M hydrochloric acid ..	0·400	$-1{\cdot}414$ mg.$^{\frac{2}{3}}$ sec.$^{-\frac{1}{2}}$ at $-0{\cdot}30$ V *vs.* S.C.E.	8·48
	3·033	do.	8·27
1·0 M hydrochloric acid ..	1·011	do.	7·95
	3·033	do.	7·80
2·0 M hydrochloric acid in 50% alcohol ..	1·011	do.	4·05
	5·055	do.	4·04

It has been noticed in the present study that in presence of 50% alcohol and potassium chloride (1 M) or potassium iodide (1 M) turbidity does not develop even after 2 hours. In these base solutions the current-voltage curves exhibit a maximum which results in a depression (inverted maximum) on addition of gelatin. This depression becomes more prominent with an increase in the concentration of pentathionate. Hence it has to be concluded that base solutions containing 50% alcohol, potassium chloride or potassium iodide are not suitable for polarographic estimations.

Discussion

1. *Split waves*

Reference to Table III indicates that when the concentration of potassium iodide is changed, the height of the total wave remains almost a constant while that of the individual waves alters. The half-wave potential of the first wave increases while that of the second wave decreases until they coalesce into a single wave. The different steps noticed do not represent different reduction states but represent the reduction of different species of tetrathionate ions in sluggish equilibrium.

The nature of the wave obtained with potassium chloride as the base electrolyte depends upon the concentration of the latter. When the concentration of tetrathionate is below 1·4 millimolar and the concentration of potassium chloride is 3 M, the diffusion current plateau is horizontal. At other concentrations of potassium chloride the diffusion current plateau is not horizontal. The incorporation of alcohol gives only one wave indicating the existence of only one ionic species.

2. *Irreversibility of polarographic waves*

Results presented in Tables I and III indicate that the tri- and tetrathionates are reduced irreversibly at the dropping mercury electrode. It is surprising that the irreversibility of the reduction process should be smaller in presence of alcohol.

In contrast to the behaviour of tri- and tetrathionates, the pentathionate gets reduced reversibly at the dropping mercury electrode as the value of $E_{\frac{1}{4}} - E_{\frac{1}{4}} = 0{\cdot}013$ V corresponding to a 4 electron reversible process.

3. *Mechanism of the reduction process*

The mechanism of the reduction process of tri- and tetrathionates cannot be deduced from the plot of log $i/(i_d - i)$ *vs.* potential as the reduction process is irreversible. Furness and Davies[2] have found that the reduction of tetrathionate corresponds to a two electron process although they

doubted very much the formation of thiosulphate by such a reaction. They have obtained a value of $9{\cdot}00\times10^{-6}$ cm.^{2} sec.$^{-1}$ for the diffusion coefficient of tetrathionate from the equivalent conductivity data at 25° C. In the present work a value of $9{\cdot}90\times10^{-6}$ cm.^{2} sec.$^{-1}$ has been calculated for 30° C. from the above value applying temperature corrections.[11] The diffusion coefficients of tri- and pentathionates have been deduced by using this value in the Northrop-Anson formula.[9]

$$D_1/D_2 = \sqrt{M_2/M_1}$$

The values for tri- and pentathionates are $1{\cdot}069\times10^{-5}$ and $9{\cdot}26\times10^{-6}$ cm.^{2} sec.$^{-1}$ It has been found by substituting these values in the equation[10]

$$607\, nD^{\frac{1}{2}} = \text{Diffusion current constant}$$

that 2, 2 and 4 electrons are involved in the reduction process of tri-, tetra- and pentathionates respectively. The following mechanism can therefore be suggested for the reduction of the thionates at the dropping mercury electrode:

$$S_3O_6^{=} + 2e \rightarrow S_2O_6^{=} + \text{Sulphide}$$

$$S_4O_6^{=} + 2e \rightarrow 2S_2O_3^{=}$$

$$S_5O_6^{=} + 4e \rightarrow S_3O_6^{=} + \text{Sulphide (highly acidic solution).}$$

A coulometric analysis could not be conducted as the reaction was complicated by the formation of mercuric sulphide.

4. *Estimation of thionates in a mixture*

(*a*) *Mixture of tri- and tetrathionates.*—The tetrathionates can be estimated readily by employing 1 M hydrochloric acid in the base solution. The total thionates can be determined by employing a base solution containing 50% alcohol, 1 M potassium chloride or iodide and 0·01% gelatin, as in this base solution the waves of the two thionates overlap. The addition of 0·01% gelatin does not decrease the diffusion current, in 50% alcohol containing potassium chloride (1 M), to any noticeable degree.

(*b*) *Estimation of a mixture of penta- and tetrathionates.*—The pentathionate can be determined in the above mixture by incorporating 50% alcohol in 2 N hydrochloric acid. In this medium, the tetrathionate does not show any reduction step. It has not been possible to estimate trithionate in presence of pentathionate as the C—V curves of pentathionate in alcoholic potassium chloride or potassium iodide solutions are characterized by a depression at about $-1{\cdot}15$ V *vs.* S.C.E.

In conclusion, it can be stated that the polarographic behaviour of thionates described in the present paper show that polarographic methods can be applied successfully for the estimation of the three thionates mentioned.

Summary

1. The polarographic behaviour of the thionates has been studied employing potassium iodide, potassium chloride, hydrochloric acid, and various buffers as electrolytes in the base solutions. The effect of incorporating 50% alcohol in the base solution has also been investigated.

2. The results indicate that the reduction process is essentially an irreversible process with tri- and tetrathionates while the opposite effect is noticed with pentathionate.

3. The tetrathionate shows the phenomenon of split wave which is practically absent in trithionate solutions.

4. A method has been suggested for the estimation of thionates in a mixture based on the use of 50% alcohol in the base solutions.

5. The mechanism of the reduction process has been discussed.

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EXPLANATION OF FIGURES

FIG. 1. Effect of concentration of potassium chloride on the polarographic behaviour of trithionate (1·703 millimolar.). Curves 1 and 2 have been taken in base solutions containing 0·1 M and 1·0 M potassium chloride. Sensitivity: *s*/70. The vertical voltage line preceding the polarogram corresponds to –0·773 V *vs.* S.C.E.

FIG. 2. Effect of alcohol on the polarographic behaviour of trithionate (1·288 millimolar) in potassium chloride solution. Curve 1 has been taken using 50% alcohol and curve 2 using 20% alcohol. Sensitivity: *s*/70. The vertical voltage line preceding the polarogram corresponds to –0·973 V *vs.* S.C.E.

FIG. 3. Effect of concentration of gelatin on the polarographic behaviour of tetrathionate (0·785 millimolar) in hydrochloric and nitric acids. Curves 1 and 2 have been taken in 0·2 M hydrochloric acid and 0·005% and 0·02% gelatin respectively, Curve 3 has been taken in 0·1 M nitric acid. Sensitivity: s/30. The vertical voltage line preceding each polarogram corresponds to +0·027 V *vs.* S.C.E.

FIG. 4. Effect of buffers on the polarographic behaviour of tetrathionate (1·048 millimolar) Curves 1, 2, 3 and 4 have been taken at pH 2·6, 4·0, 2·6 and 2·6 respectively. Curves 1 and 2 have been taken using hydrochloric acid and sodium acetate buffers, while curves 3 and 4 were taken with disodium hydrogen phosphate-citric acid buffers. To the base solutions under which curves 2 and 4 were taken, extra sodium chloride was added to keep the concentration of sodium ion as in experiment 1. Sensitivity: s/30. The vertical voltage line preceding each polarogram corresponds to +0·027 V *vs.* S.C.E.

FIG. 5. Effect of concentration of potassium iodide on the polarographic behaviour of tetrathionate (1·307 millimolar). Curves 1, 2, 3, 4 and 5 have been taken with the following concentrations of potassium iodide: 3·00 M, 1·00 M, 0·50 M, 0·04 M, 0·016 M respectively. Sensitivity: *s*/70. The vertical voltage line preceding each polarogram corresponds to –0·373 V *vs.* S.C.E.

FIG. 6. Effect of concentration of potassium iodide on the polarographic behaviour of tetrathionate (1·307 millimolar). Curves 1, 2, 3 and 4 have been taken with the following concentrations of potassium iodide: 0·30, 0·16, 0·08 and 0·06 Molar respectively. Sensitivity: *s*/70. The vertical voltage line preceding each polarogram corresponds to –0·373 V *vs.* S.C.E.

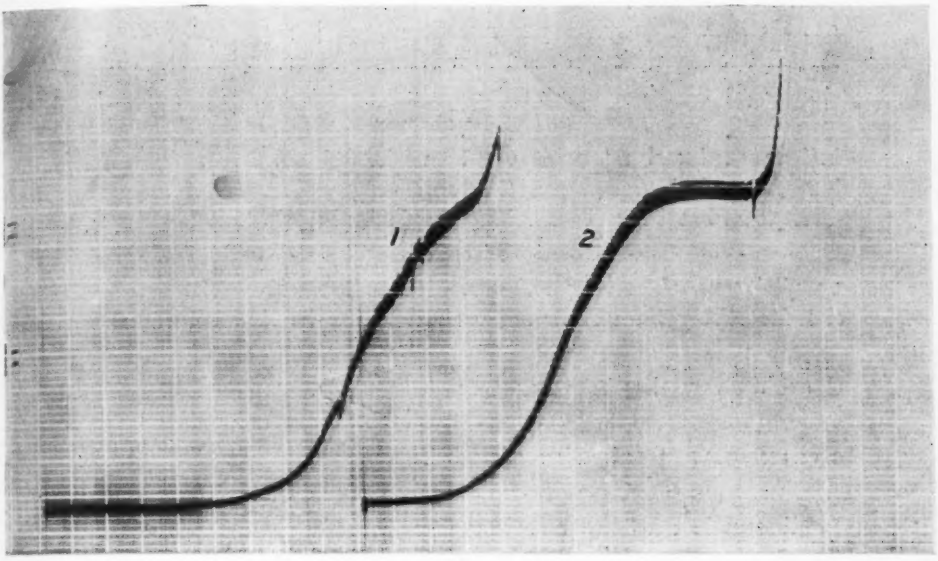
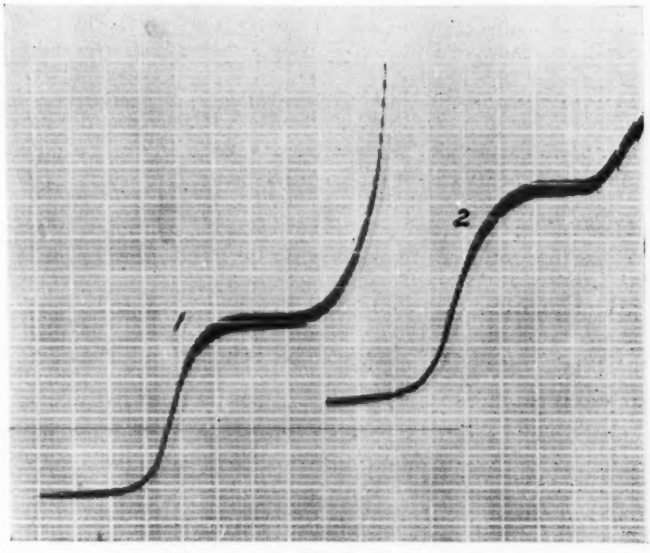


FIG. 1



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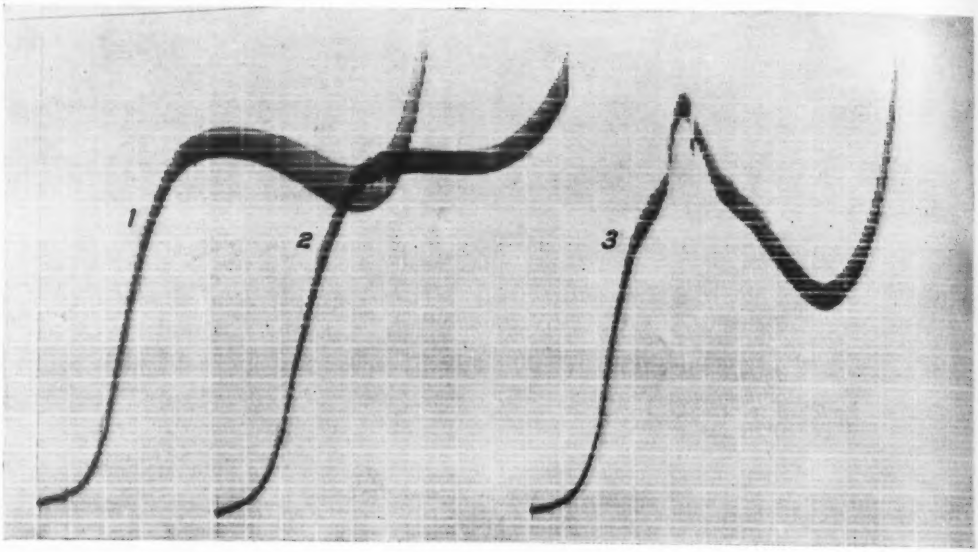

FIG. 3

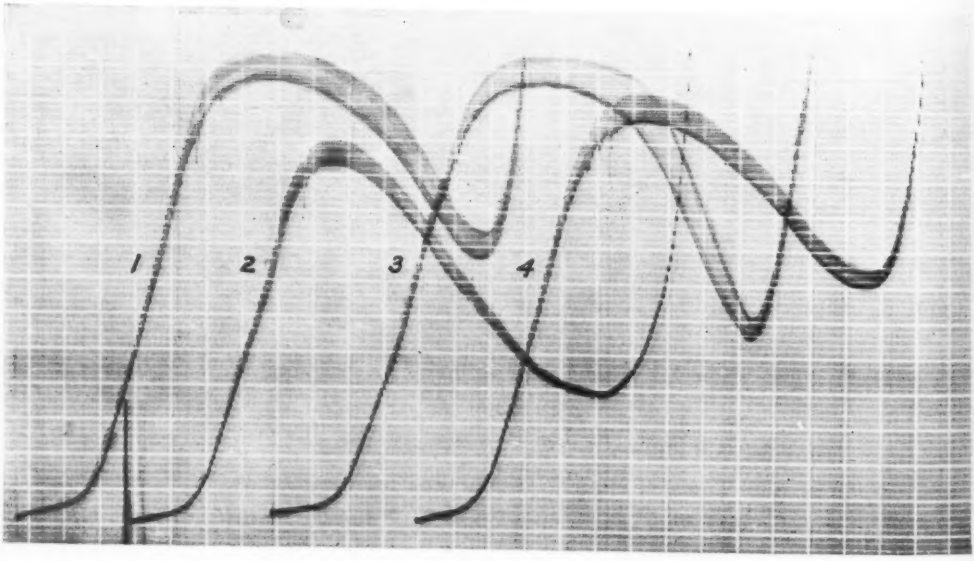

FIG. 4

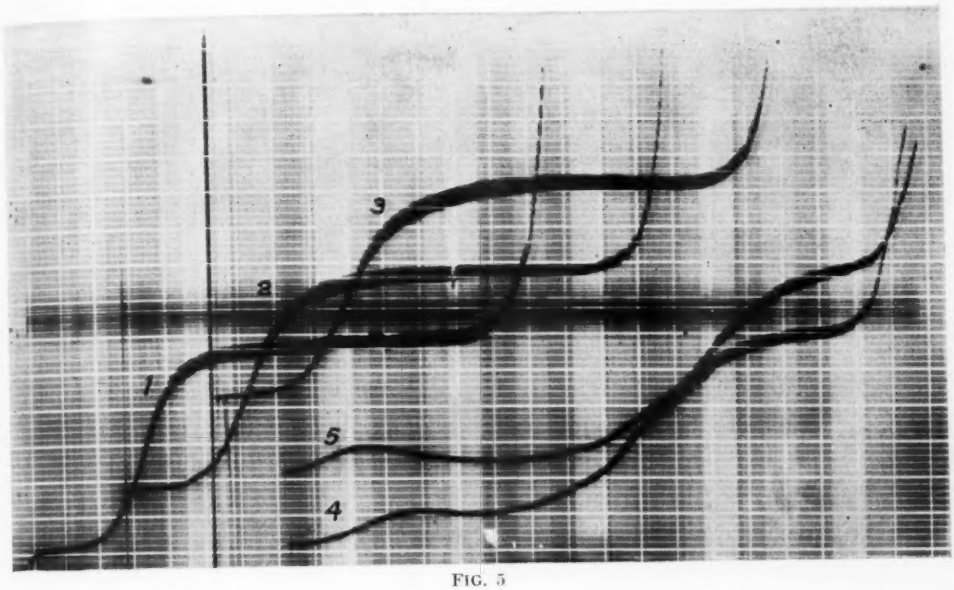

FIG. 5

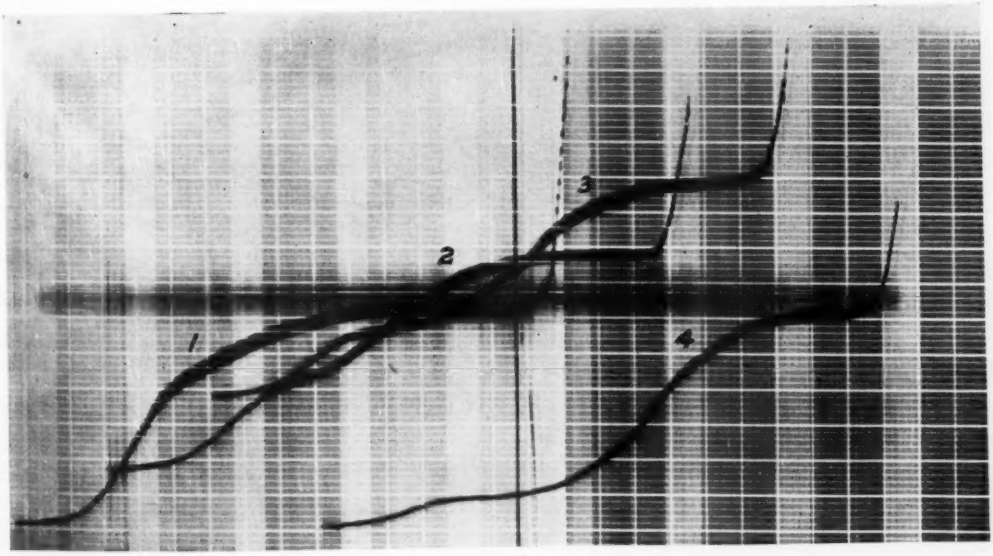

FIG. 6

C-METHYLATION OF PHLOROGLUCINOL DERIVATIVES

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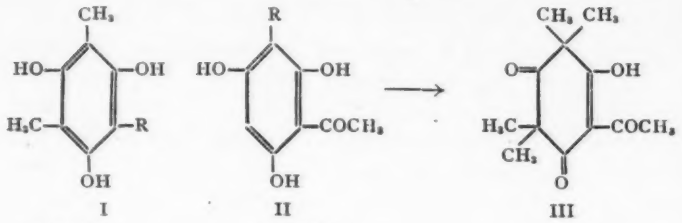
Among naturally occurring C-methyl flavonoids, a few of them are known to have C-methyl groups in both 6- and 8-positions, *e.g.*, matteucinol, desmethoxy-matteucinol and angustifolionol. Their syntheses require 3:5-dimethyl phloracetophenone (I *b*) as the starting material. Though this ketone is readily obtained by the Hoesch reaction on 3:5-dimetyl phloroglucinol (I *a*),[1, 2] the phenol is difficult to obtain and requires a number of steps.[3, 4] The nuclear methylation of C-methyl phloracetophenone (II *a*) which possesses the structural requirements for C-methylation[5] would be more direct. Though it does not take place with methyl iodide and potassium carbonate in acetone medium,[6] in view of the resemblance in structure to resacetophenone, the reaction was carried out using excess of methanolic potash or sodium methoxide. It however yielded a different product which was a low melting solid, gave an intense orange-red ferric reaction and was soluble in aqueous sodium bicarbonate. It did not couple with diazotised *p*-nitraniline and also did not undergo any change when heated with aluminium chloride in benzene solution showing that it was not a methyl ether. These properties and analytical data can only be explained on the basis that it is 5-acetyl-1:1:3:3-tetramethylcyclohexen-(4)-ol-(4)-dione (2 : 6) (III). The same product was obtained when phloracetophenone (II *b*) itself was subjected to nuclear methylation under the same conditions. The corresponding formyl compound (IV) was reported earlier to be produced by heating under reflux 3:5-dimethyl phloroglucinaldehyde (I *c*) with methyl iodide and methanolic potash.[7] 3:5-Dimethyl compound may therefore be considered to be a possible intermediate stage in the nuclear methylation of carbonyl derivatives of phloroglucinol though the exact conditions for its preparation are not known. This conclusion is also supported by an earlier observation of Herzig *et al.*[8] that silver phloroglucinol carboxylate on heating with excess of methyl iodide in a sealed tube gives methyl-3:5-dimethyl phloroglucinol carboxylate along with other products.

The structure (III) for the nuclear methylation product of C-methyl phloracetophenone has now been further supported by the nuclear methylation of phloroisovalerophenone (V) under the same conditions when lepto-



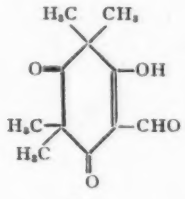
I

a, R=H
b, R=$COCH_3$
c, R=CHO

II

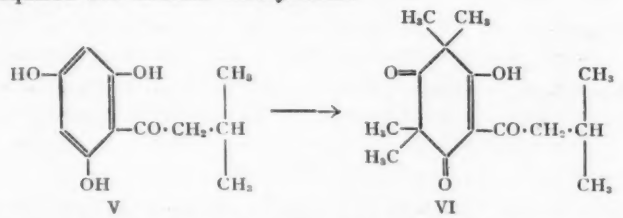
a, R=CH_3
b, R=H

III



IV

spermone which occurs in the oil of *Leptospermum flavescens*[9] and possesses an analogous structure (VI)[10, 11] could be obtained in 65% yield. It was earlier synthesised by Briggs *et al.*[12] using rather vigorous conditions. They heated a mixture of the phloroisovalerophenone (V), methyl iodide and aqueous potash in a sealed tube at 70° for 3 days and obtained a comparatively less yield (32%) of leptospermone (VI). Thus it is clear that carbonyl derivatives of phloroglucinol differ markedly from those of resorcinol which uniformly give the 3-C-methyl-4-O-methyl derivatives under similar conditions of nuclear methylation (see Ref. 5). This difference in behaviour seems to lie in their difference in capacity to assume the ketonic form required for nuclear methylation.

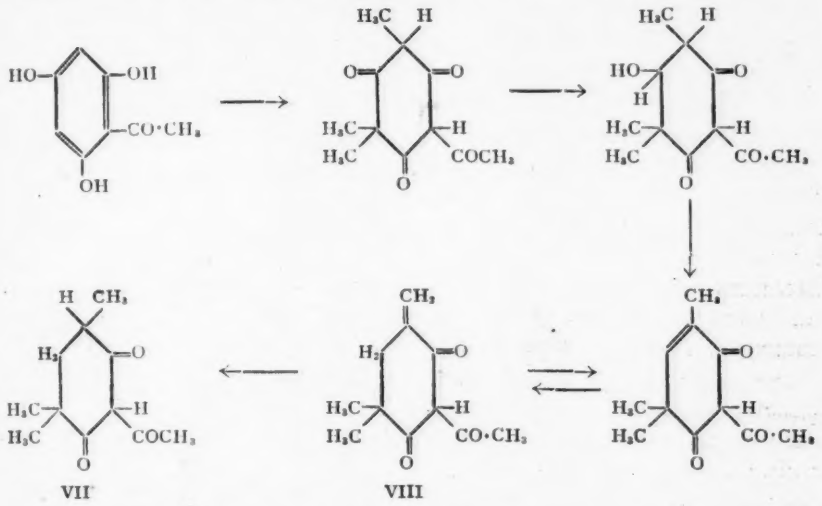


V

VI

C-Polymethylation in a phloroglucinol derivative seems to be a natural process as a number of C-polymethylated β-triketones are known to occur in nature. Important examples are: leptospermone (VI), protokosin, α-kosin and β-kosin found in the anthelmintic drug, kousso (*Hagenia abyssinica*)[13, 14] and a number of substances from male and female ferns

(*Aspidium filix*) among which aspidin,[14] aspidinol,[15] albaspidin[14] and flavaspidic acid[15] are more important. There are two other C-polymethylated compounds but they are β-diketones; angustione (VII) and dehydroangustione (VIII) occurring in admixture in the oil of *Backhousia angustifolia*.[16–19] These two also seem to be evolved in nature from a phloroglucinol derivative, phloracetophenone (the dimethyl ether of which is found in nature).[20] The various stages in the evolution may be C-trimethylation, reduction, elimination of water (dehydroangustione VIII) and further reduction of the double bond (angustione VII) as shown below:



On the expectation that milder conditions may give appreciable yields of 3:5-dimethyl phloracetophenone, experiments using lesser proportions of methanolic potash have been conducted. From the resulting mixtures it could be isolated in 2% yield when 2 moles of alkali and in 5% yield when 3 moles of alkali are used. The use of 1 mole of alkali gives rise to a 50% yield of 3-methyl-phloracetophenone (II *a*) and 3 moles of alkali yields mainly 5-acetyl-1:3:3-trimethyl cyclohexen-(4)-ol-(4)-dione (2:6).

Experimental

5-Acetyl-1:1:3:3-tetramethyl-cyclohexen-(4)-(ol)-4-dione-(2:6) (III):

(i) *By the nuclear methylation of 3-methyl phloracetophenone*

With methanolic potash.—To an ice-cooled solution of the ketone (2 g.) in methanolic potash (3 g. in 20 c.c.) methyl iodide (5 c.c.) was added in

one lot and the whole solution well shaken and kept overnight at room temperature. It was then refluxed with more of methanolic potash (1·5 g. in 10 c.c.) and methyl iodide (2·5 c.c.) for 6 hours. The solvent was removed under vacuum and dilute hydrochloric acid added to acidify the mixture. On cooling a soid separated. It was filtered, washed with water and triturated with aqueous sodium bicarbonate. Most of it dissolved, leaving behind a small amount of fluffy material. The bicarbonate solution was acidified and the colourless solid so obtained crystallised from aqueous methanol, yielding colourless needles, m.p. 52–53°. Yield, 1·8 g. It gave an intense orange-red colour with alcoholic ferric chloride, did not couple with diazotised *p*-nitraniline and also did not undergo any change when heated with aluminium chloride in benzene solution for two hours (Found: C, 64·3; H, 7·2; $C_{12}H_{16}O_4$ requires C, 64·3; H, 7·1%).

Equal amounts of *p*-toluidine and the above product were heated on a boiling water-bath for half an hour and then refluxed for 5 minutes. The product was poured into ice-water, extracted with ether and the ether solution washed with dilute hydrochloric acid, followed by aqueous sodium hydroxide and water. The ether was removed and the residue was crystallised from 50% alcohol when the *p*-toluidino compound separated as pale yellow needles melting at 134° (Found: C, 73·1; H, 7·4; $C_{19}H_{23}O_3N$ requires C, 72·8; H, 7·3%).

With sodium methoxide.—C-Methyl phloracetophenone (2 g.) was dissolved in methanolic sodium methoxide (2 g. sodium in 30 c.c.) and refluxed for 3 hours with excess of methyl iodide (10 c.c.). The solvent was distilled off and the product was worked up as described in the above experiment. It melted at 52–53° and was identical with the sample obtained by using alcoholic potash (1·3 g.).

(ii) *By the nuclear methylation of phloracetophenone*

Phloracetophenone (2 g.) was methylated by both methods and the same product was formed melting at 52–53° and identical in all respects with the nuclear methylation product of C-methyl phloracetophenone. Yield, 1·6 g.

Leptospermone (*Nuclear methylation of phloroisovalerophenone*).—Phloroisovalerophenone[21] (2 g.) was dissolved in methanolic sodium methoxide (1·5 g. of sodium in 20 c.c.) and then refluxed for 3 hours with excess of methyl iodide (10 c.c.) . The solvent was distilled off under vacuum and dilute hydrochloric acid added. The product was extracted with ether and the ether solution washed with a dilute aqueous solution of sodium sulphite (5 g.). It was dried over anhydrous sodium sulphate. The residue left

after evaporation of ether was distilled under 10 mm. pressure (bath temp. 120–40°). Yield, 1·6 g. It was soluble in aqueous sodium carbonate and gave an orange-red colour with alcoholic ferric chloride. The *p*-toluidino compound prepared in the same way as described for an earlier case crystallised from aqueous alcohol in the form of colourless needles melting at 100–01° (Found: C, 74·6; H, 8·4; $C_{22}H_{29}O_3$ requires C, 74·4; H, 8·2%). In all these properties the product agreed with leptospermone described by Briggs *et al.*[11, 12]

Partial Methylation of Phloracetophenone.—(i) To a boiling solution of phloracetophenone (3 g.) in methanol (10 c.c.) was added methyl iodide (6 c.c., excess) in one lot and methanolic potash (10%, 10 c.c.; 1 mole) gradually in the course of 3 hours. The product was taken up in ether as described earlier and extracted successively with 5% aqueous solutions of sodium bicarbonate (*a*), sodium carbonate (*b*) and sodium hydroxide (*c*). The remaining ether solution contained no substance; (*a*) was negligible; (*b*) gave C-methyl-phloracetophenone (50%), m.p. 210–11° after crystallisation from boiling water, acetate, m.p. 111°; (*c*) yielded 2·5% of C-methyl-phloracetophenone dimethyl ether, m.p. 140–41°.

(ii) Same as above using 20 c.c. (2 moles) of methanolic potash: (*a*) gave 10% of 5-acetyl-1 : 3 : 3-trimethyl cyclohexen-(4)-ol-(4)-dione (2 : 6), m.p. 161–62° (from 30% methanol) which underwent deacetylation on boiling with 7% hydrochloric acid for four hours to 3-methyl filicinic acid, m.p. 180–81°; (*b*) 3 : 5-dimethyl-phloracetophenone (2%), m.p. 220–22° from 30% methanol and 3-methyl-phloracetophenone (20%) from boiling water.

(iii) Same as above using 30 c.c. (3 moles) of methanolic potash: (*a*) yielded on fractional crystallisation from 30% methanol, 5-acetyl-1 : 3 : 3-trimethyl-cyclohexen-(4)-ol-(4)-dione (2 : 6) (25%), m.p. 161–62°; (*b*) gave 3 : 5-dimethyl phloracetophenone (5%) from 30% methanol and C-methyl phloracetophenone (15%) from boiling water.

Summary

Nuclear methylation of phloracetophenone and 3-C-methyl phloracetophenone, under vigorous conditions yields 5-acetyl-1 : 1 : 3 : 3-tetramethyl cyclohexen-(4)-ol-(4)-dione (2 : 6) (III); the intermediate 3 : 5-dimethyl compound could not be obtained. This method is convenient for the preparation of leptospermone (VI). Many natural products seem to be formed by the nuclear methylation of the carbonyl derivatives of phloroglucinol. Using lesser proportions of alcoholic potash lower methylation products are obtained; but 3 : 5-dimethyl-phloroacetophenone could be isolated only in small quantities.

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CONTENTS

PAGE

Studies on Collagen. I. Structure of the Collagen Group of Proteins . . . . . G. N. Ramachandran and Gopinath Kartha 215

The Propagation of Light in Absorbing Biaxial Crystals. II. Experimental . . . . . S. Pancharatnam 235

Search for Physiologically Active Compounds. Part 1. Synthesis of 7-Amino and 7-Halo-4-methyl-3-phenyl Coumarins . . . . . N. V. Subba Rao and V. Sundaramurthy 249

Synthetic Experiments in the Benzopyrone Series. Part LVII. Synthesis of 5: 6: 7: 8: 4′-Pentamethoxy Flavanone and Flavone: The Constitution of Ponkanetin . . . . J. M. Sehgal, T. R. Seshadri and K. L. Vadehra 252

Thermal Expansion of Potassium Iodide . . . . . R. Srinivasan 255

Studies in the Isoquinoline Series. Part II. Oxidation of 1-Benzyl-3: 4-Dihydroisoquinolines . . . T. R. Govindachari and K. Nagarajan 261

Polarographic Behaviour of Thionates . . . . . R. S. Subrahmanya 267

C-Methylation of Phloroglucinol Derivatives . . . . . A. C. Jain and T. R. Seshadri 279